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AETE

AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

PROGRAMME D'ÉVALUATION DES TECHNIQUES DE MESURE D'IMPACTS EN MILIEU AQUATIQUE

AETE Synthesis Report of Selected Technologies for Cost-Effective Environmental Monitoring of Mine Effluent Impacts in Canada

AETE Project 4.1.4

Aquatic Effects Program ✓ Programme des Effets Aquatiques

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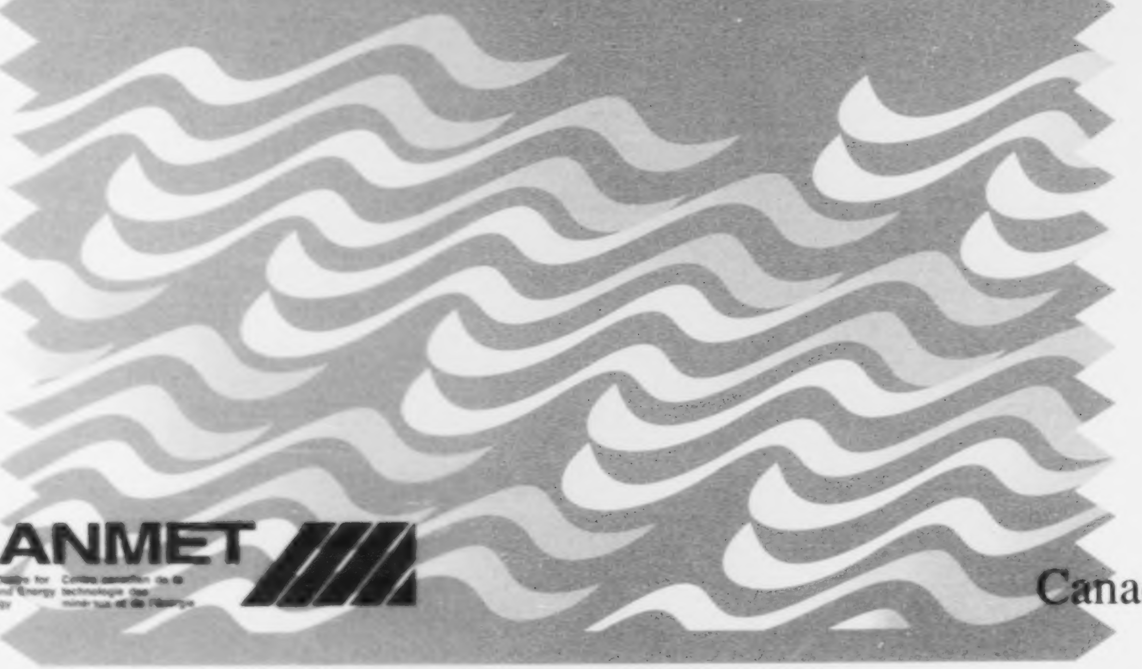

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Canada Centre for Mineral and Energy Technology
Centre canadien de la technologie des minéraux et de l'énergie

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AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE) PROGRAM

AETE Synthesis Report of Selected Technologies for Cost-Effective Environmental Monitoring of Mine Effluent Impacts in Canada

AETE Project 4.1.4

AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE) PROGRAM SYNTHESIS REPORT OF SELECTED TECHNOLOGIES FOR COST-EFFECTIVE ENVIRONMENTAL MONITORING OF MINE EFFLUENT IMPACTS IN CANADA

(AETE SYNTHESIS REPORT)

Prepared for:

Canada Centre for Mining and Energy Technology
555 Booth Street
Ottawa, Ontario
K1A 0G1
and
Mining Association of Canada
350 Sparks Street
Ottawa, Ontario
K1R 7S8

Prepared by:

ESG International Inc.
361 Southgate Drive
Guelph, Ontario
N1G 3M5

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AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

Notice to Readers

AETE Synthesis Report of Selected Technologies for Cost-Effective Environmental Monitoring of Mine Effluent Impacts in Canada

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, several federal government departments and a number of provincial governments; it is coordinated by the Canada Centre for Mineral and Energy Technology (CANMET). The program was designed to be of direct benefit to the industry, and to government. Through technical and field evaluations, it identified cost-effective technologies to meet environmental monitoring requirements. The program included three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring.

The technical evaluations are conducted to document certain tools selected by AETE members, and to provide the rationale for doing a field evaluation of the tools or provide specific guidance on field application of a method. In some cases, the technical evaluations included a go/no go recommendation that AETE takes into consideration before a field evaluation of a given method is conducted.

The technical evaluations are published although they do not necessarily reflect the views of the participants in the AETE Program. The technical evaluations should be considered as working documents rather than comprehensive literature reviews. The purpose of the technical evaluations was to document specific monitoring tools. AETE committee members would like to stress that no one single tool can provide all the information required for a full understanding of environmental effects in the aquatic environment.

Any comments concerning the content of this report should be directed to:

Geneviève Béchard
Manager, Metals and the Environment Program
Mining and Mineral Sciences Laboratories - CANMET
Room 330, 555 Booth Street, Ottawa, Ontario, K1A 0G1
Tel.: (613) 992-2489 Fax: (613) 992-5172
E-mail: gbechard@nrcan.gc.ca

PROGRAMME D'ÉVALUATION DES TECHNIQUES DE MESURE D'IMPACTS EN MILIEU AQUATIQUE

Avis aux lecteurs

Programme ETIMA - Rapport de synthèse sur des méthodes de surveillance choisies permettant d'évaluer dans une perspective coût-efficacité les effets des effluents miniers sur les écosystèmes aquatiques au Canada

Le Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ÉTIMA) visait à évaluer les différentes méthodes de surveillance des effets des effluents miniers sur les écosystèmes aquatiques. Il est le fruit d'une collaboration entre l'industrie minière du Canada, plusieurs ministères fédéraux et un certain nombre de ministères provinciaux. Sa coordination relève du Centre canadien de la technologie des minéraux et de l'énergie (CANMET). Le programme était conçu pour bénéficier directement aux entreprises minières ainsi qu'aux gouvernements. Par des évaluations techniques et des études de terrain, il a permis d'évaluer et de déterminer, dans une perspective coût-efficacité, les techniques qui permettent de respecter les exigences en matière de surveillance de l'environnement. Le programme comportait les trois grands volets suivants : évaluation de la toxicité aiguë et sublétale, surveillance des effets biologiques des effluents miniers en eaux réceptrices, et surveillance de la qualité de l'eau et des sédiments.

Les évaluations techniques ont été menées dans le but de documenter certains outils de surveillance sélectionnés par les membres de l`ÉTIMA et de fournir une justification pour l`évaluation sur le terrain de ces outils ou de fournir des lignes directrices quant à leur application sur le terrain. Dans certains cas, les évaluations techniques pourraient inclure des recommandations relatives à la pertinence d`effectuer une évaluation de terrain que les membres de l`ÉTIMA prennent en considération.

Les évaluations techniques sont publiées bien qu`elles ne reflètent pas nécessairement toujours l'opinion des membres de l`ÉTIMA. Les évaluations techniques devraient être considérées comme des documents de travail plutôt que des revues de littérature complètes. Les évaluations techniques visent à documenter des outils particuliers de surveillance. Toutefois, les membres de l`ÉTIMA tiennent à souligner que tout outil devrait être utilisé conjointement avec d`autres pour permettre d`obtenir l`information requise pour la compréhension intégrale des impacts environnementaux en milieu aquatique.

Les personnes intéressées à faire des commentaires concernant le contenu de ce rapport sont invitées à communiquer avec M^me^ Geneviève Béchard à l'adresse suivante :

Geneviève Béchard
Gestionnaire, Programme des métaux et de l'environnement
Laboratoires des mines et des sciences minérales - CANMET
Pièce 330, 555, rue Booth, Ottawa (Ontario), K1A 0G1
Tél.: (613) 992-2489 / Fax : (613) 992-5172
Courriel : gbechard@nrcan.gc.ca

EXECUTIVE SUMMARY

The Canadian Government committed to review the *Metal Mining Liquid Effluent Regulations* (MMLERs) in the early 1990's. Discussions established the need to identify tools that could be used to monitor the effects of effluents from mining operations on the aquatic environment. This provided the impetus for the Aquatic Effects Technology Evaluation (AETE) program.

The mandate for the AETE program was twofold:

1) to evaluate environmental monitoring technologies that could be used to assess the impacts of mine effluents, and
2) to recommend specific methods or groups of methods that will permit accurate characterization of environmental impacts in as cost-effective a manner as possible.

The AETE program was a joint initiative of the Canada Centre for Mineral and Energy Technology (CANMET) and the Mining Association of Canada (MAC). Other federal and provincial departments participated on the management and technical committees. The $3.4M program ran from 1994 to 1998 and supported over 30 individual studies and projects. This Synthesis Report provides a summary and overview of the program, major findings and final recommendations.

The program consisted of three main technical areas:

a) acute and sublethal toxicity test methods,
b) water and sediment monitoring methods, and
c) biological monitoring methods in receiving waters

The tools and methods recommended in this report should be suitable for use in a routine monitoring program to document existing environmental conditions and to determine if there is a measurable effect. The federal government is also developing an Environmental Effects Monitoring (EEM) program for the mining sector in Canada under the federal *Fisheries Act*. The AETE Synthesis Report provides a list of recommended tools that could be adopted in the EEM program.

Monitoring tools considered by the AETE program had to address at least one of the following guiding questions:

1) are contaminants getting into the system?
2) are contaminants bioavailable?
3) is there a measurable biological response?
4) are the contaminants in the system causing the observed response?

Over 100 potential monitoring tools were considered through initial screening, literature (technical) reviews, laboratory and field testing. Tools were evaluated using several criteria including ability to answer one or more of the four guiding questions, demonstrated performance in the AETE program, cost, practicality and availability of standardized protocols.

Only tools evaluated within the context of AETE were recommended, although it is recognized that other monitoring methods may be available for more detailed studies. Furthermore, although some tools were not considered suitable for use in routine monitoring programs, their potential application in detailed site-specific investigations is recognized by AETE.

The toxicity testing component examined five rapid micro-toxicity tests as potential alternatives to the commonly used Rainbow trout and *Daphnia magna* acute lethality tests for examination of liquid effluents. Secondly, nine sublethal toxicity test methods were evaluated for their ability to detect sublethal effluent effects. In addition, the ability of sublethal toxicity test results to predict downstream biological effects was examined. Four sediment toxicity test methods were also evaluated to determine their ability to detect effects from mine discharges.

The mandate of the water and sediment monitoring component of AETE was to evaluate methods of assessing effects of mine effluents to receiving waters and their underlying sediments. The tools and methods evaluated included total versus dissolved metal concentrations in water, filtering methods, analytical detection limits, methods of sediment collection and analysis of total or partial metal levels in sediments.

The biological monitoring component evaluated tools to determine if contaminants were bioavailable, and to measure biological responses in receiving waters. A wide range of tools were considered including metal levels and biochemical indices in plant and animal tissues, metallothionein levels in fish tissues, various measures of benthic invertebrates and surveys of fish including abundance, growth, histopathology, reproduction and organ size.

A series of thirteen formal hypotheses were also developed that could be tested during field studies conducted in 1997. The hypotheses were based on the four guiding questions but helped clarify comparisons of individual monitoring tools (e.g. total versus dissolved metals in water). In addition, a number of integration hypotheses were developed to examine the relationships between different tools or monitoring results (e.g. relationship between sediment toxicity and benthic invertebrates).

Field trials of the candidate tools comprised a significant portion of the AETE program during three different years. A pilot program was conducted in 1995 at one mine site to evaluate 10 specific monitoring tools. The 1996 field program included a preliminary evaluation of seven (7) mine sites across Canada that incorporated toxicity testing as well as environmental measurements. The purpose of the 1996 program was to select four mine sites for further detailed testing, and to prepare a study design for the detailed investigations. The criteria to select four sites for detailed evaluation included:

- presence of a well defined gradient of water, sediment, toxicity and biological effects
- availability of adequate reference stations, and
- suitability of the site to test hypotheses

The 1997 field program included two phases:

1. detailed field and laboratory evaluation and hypothesis testing at four mine sites, and
2. data interpretation and comparative assessment of the monitoring methods.

To examine the question "*are the contaminants in the system causing the observed response?*", a weight-of-evidence approach was investigated. Specifically, the sediment quality triad was used to examine the statistical relationships between water and sediment chemistry, toxicity and in-stream (or lake) biological responses.

The various technical evaluations, laboratory testing and field surveys all produced individual reports for the AETE program that included recommendations pertaining to monitoring tools. Where there were discrepancies between a technical review and the field survey observations, greater weight was given to the field results when considering a recommendation. In the end, only the tools recommended in this Synthesis Report are endorsed by AETE. Sixteen individual tools are recommended as suitable candidates in a routine mine monitoring program. An additional six tools are recognized as having potential application in more detailed site specific investigations.

RÉSUMÉ

Le gouvernement du Canada s'est engagé à revoir le *Règlement sur les effluents liquides des mines de métaux* (RELMM) au début des années 90. Des discussions ont permis d'établir le besoin d'identifier les outils qui pourraient servir à surveiller les effets des effluents des opérations minières sur le milieu aquatique. Cela a fourni l'élan nécessaire au Programme d'évaluation des techniques de mesure d'impact en milieu aquatique (ETIMA).

Le mandat du programme ETIMA était composé de deux volets :

1) évaluer les technologies de surveillance environnementale qui pourraient être utilisées pour évaluer les impacts des effluents miniers, et

2) recommander des méthodes ou groupes de méthodes spécifiques qui permettront de caractériser de façon précise les incidences environnementales le plus économiquement possible.

Le programme ETIMA était une initiative conjointe du Centre canadien de la technologie des minéraux et de l'énergie (CANMET) et de l'Association minière du Canada (AMC). D'autres ministères fédéraux et provinciaux ont participé aux comités de gestion et de technique. Le programme, doté d'un budget de 3,4 millions de dollars, s'échelonnait de 1994 à 1998, et supportait plus de 30 études et projets individuels. Le présent rapport de synthèse fournit un résumé et un aperçu du programme, des principales conclusions et des recommandations finales.

Le programme comprenait trois principaux domaines techniques :

a) les méthodes de test de toxicité aiguë et sublétale,

b) les méthodes de surveillance de l'eau et des sédiments, et

c) les méthodes de surveillance biologique des eaux réceptrices.

Les outils et les méthodes recommandés dans ce rapport devraient pouvoir être utilisés dans un programme de surveillance de routine afin de documenter les conditions environnementales existantes et de déterminer s'il y a un effet mesurable. Le gouvernement fédéral met également au point un Programme de suivi des effets sur l'environnement (SEE) pour le secteur minier au Canada en vertu de la *Loi sur les pêches* fédérale. Le rapport de synthèse du programme ETIMA donne une liste des outils recommandés qui pourraient être adoptés dans le programme SEE.

Les outils de surveillance pris en compte par le programme ETIMA devaient porter sur au moins une des questions suivantes :

1) est-ce que les contaminants pénètrent dans le réseau aquatique?

2) les contaminants sont-ils biodisponibles?

3) la réponse (biologique) est-elle mesurable?

4) les contaminants dans le réseau sont-ils la cause de cette réponse?

Plus d'une centaine d'outils de surveillance possibles ont été pris en compte lors d'un tri initial, d'examens de la littérature (technique), et de tests en laboratoire et sur le terrain. Les outils ont été évalués à l'aide de plusieurs critères, notamment l'aptitude à répondre à une ou plusieurs des questions, la performance démontrée dans le programme ETIMA, le coût, le côté pratique et la disponibilité pour les protocoles normalisés.

Seuls les outils évalués dans le cadre du programme ETIMA ont été recommandés, bien que l'on reconnaisse que d'autres méthodes de surveillance puissent être disponibles en vue d'études plus détaillées. De plus, même si certains outils n'étaient considérés comme aptes à une utilisation dans des programmes de surveillance de routine, le programme ETIMA a reconnu leur application possible dans des études détaillées propres au site.

La composante « test de toxicité » a examiné cinq tests rapides de micro-toxicité comme remplaçants possibles des tests de létalité aiguë sur le truite arc-en-ciel et *Daphnia magna* communément utilisés pour examiner les effluents liquides. On a ensuite évalué la capacité de neuf méthodes de tests de toxicité sublétale à déceler les effets de l'effluent sublétal. De plus, on a examiné l'aptitude des résultats du test de toxicité sublétale à prévoir les effets biologiques en aval. On a également évalué quatre méthodes de test de toxicité des sédiments afin de déterminer leur aptitude à déceler les effets des rejets des mines.

Le mandat de la composante « surveillance de l'eau et des sédiments » du programme ETIMA était d'évaluer les méthodes d'évaluation des effets des effluents miniers sur les eaux réceptrices et leurs sédiments. Les outils et méthodes étudiés comprenaient les concentrations de métal total versus dissous dans l'eau, les méthodes de filtration, les limites de détection analytique, les méthodes de collecte des sédiments et l'analyse des concentrations de métal total ou partiel dans les sédiments.

La composante « surveillance biologique » évaluait les outils afin de déterminer si les contaminants étaient biodisponibles, et de mesurer les réponses biologiques dans les eaux réceptrices. Une vaste gamme d'outils ont été pris en compte, notamment les concentrations de métaux et les indices biochimiques dans les tissus des plantes et des animaux, les teneurs en métallothionéine dans les tissus des poissons, diverses mesures des invertébrés benthiques et des études des poissons, dont l'abondance, la croissance, l'histopathologie, la reproduction et la taille des organes.

On a également formulé une série de treize hypothèses officielles qui ont pu être vérifiées lors d'études faites sur le terrain en 1997. Les hypothèses étaient basées sur quatre questions, mais elles ont permis de clarifier des comparaisons entre certains outils de surveillance (p. ex. métaux totaux versus dissous dans l'eau). De plus, on a formulé un certain nombre d'hypothèses d'intégration afin d'examiner les rapports entre les différents outils ou résultats de surveillance (p. ex. rapport entre la toxicité des sédiments et les invertébrés benthiques).

Les essais sur le terrain des outils retenus comprenaient une grande partie du programme ETIMA durant trois années. En 1995, on a mené un programme pilote sur un site minier dans le but d'évaluer 10 outils de surveillance spécifiques. Le programme de 1996 sur le terrain comprenait une évaluation préliminaire de sept (7) sites miniers canadiens qui incorporaient les tests de toxicité ainsi que les mesures environnementales. Le but du programme de 1996 était de choisir quatre sites miniers en vue d'un essai plus détaillé, et de préparer un plan d'étude en vue d'enquêtes détaillées. Les critères utilisés pour choisir les quatre sites pour une évaluation détaillée étaient :

- la présence d'un gradient bien défini de l'eau, des sédiments, de la toxicité et des effets biologiques;
- la disponibilité de stations témoins adéquates ;
- la compatibilité du site aux hypothèses de l'essai.

Le programme sur le terrain de 1997 comprenait deux phases :

1. une évaluation détaillée sur le terrain et en laboratoire et une vérification des hypothèses à quatre sites miniers, et
2. l'interprétation des données et une évaluation comparative des méthodes de surveillance.

Afin d'examiner la question « *les contaminants du réseau sont-ils la cause de cette réponse?* », on a procédé par l'approche du « poids de la preuve ». De façon plus spécifique, on a utilisé la triade de la qualité des sédiments afin d'examiner les rapports statistiques entre la chimie de l'eau et des sédiments, la toxicité et les réponses biologiques dans le cours d'eau (ou le lac).

Les divers essais en laboratoire, évaluations techniques et études sur le terrain ont tous fait l'objet de rapports individuels pour le programme ETIMA, qui comprenaient les recommandations propres aux outils de surveillance. Lorsqu'il y avait des différences entre un examen technique et les observations faites dans le cadre de l'étude sur le terrain, on accordait une plus grande importance aux résultats de terrain lorsqu'on examinait une recommandation. À la fin, seuls les outils recommandés dans le Rapport de synthèse sont endossés par l'ETIMA. Seize outils individuels ont été recommandés comme candidats potentiels dans un programme de surveillance minière de routine. Six autres outils sont reconnus comme pouvant avoir une application possible dans des enquêtes plus détaillées propres au site.

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1. INTRODUCTION

1.1 Background

In 1990, Environment Canada and the Department of Fisheries and Oceans committed to re-examine the *Metal Mining Liquid Effluent Regulations* (MMLER). In 1992, Environment Canada sponsored a workshop to discuss the MMLER revision process with all stakeholders that have an interest in mining and the environment.

The initial discussions among the various stakeholders identified the need to thoroughly review and evaluate the existing MMLERs. This need provided the foundation for the AETE (Aquatic Effects Technology Evaluation) and AQUAMIN (Aquatic Effects of Mining in Canada) programs described in Sections 1.2 and 1.3, respectively.

The MMLERs were promulgated in 1977 under the authority of the *Fisheries Act*. The objective of the MMLER was to limit the discharge of deleterious substances into waters frequented by fish from new, expanded and re-opened (since 1977) base metal, uranium, and iron ore mines. The regulations set authorized concentration limits for the following parameters: arsenic, copper, lead, nickel, radium-226, total suspended solids, zinc and the lower pH limit. The concentration limits are "technology based," with the application of best practicable technology (BPT).

For the purposes of the regulations, "effluent" includes mine water effluent, mill process effluent, effluent from tailings, treatment pond effluent or treatment facility effluent, as well as seepage and surface drainage from the site. Operators are required to measure or estimate the volume of effluent discharged. The regulations do not apply to mines opened before February, 1977. During the past 25 years, the number of operating metal mines in Canada at any given time has ranged from 103 to 177.

The MMLER themselves do not require acute lethality testing, and do not require that effluents be non-acutely lethal, but the *Guidelines for the Measurement of Acute Lethality in Liquid Effluents from Metal Mines*, accompany the MMLER. These guidelines currently require only the rainbow trout pass/fail bioassay test. If 50% of the fish survive exposure to undiluted effluent for 96 hours, the effluent is considered to have passed the test.

The regulations also do not apply to gold mines, which are defined as mines where the gold produced is recovered at the site by cyanidation and accounts for more than 50% of the value of the mine's output. In the mid-1970s, there were few treatment methods in general use for controlling cyanide-bearing wastes from gold mines. Untreated cyanide-bearing effluents are generally toxic to fish; however, cyanide treatment technologies are now in place at all gold mines in Canada.

Effluent quality objectives are not legally enforceable for gold mines, but such operations are subject to the general provisions of the *Fisheries Act*. Compliance with the guidelines is considered to meet the spirit of the law. A mine may also be legally obligated to meet the guidelines if a territorial or provincial government agency imposes the limits in a permit or license issued under its legislation.

1.2 AETE Program Mandate

The AETE (Aquatic Effects Technology Evaluation) program was a joint initiative of CANMET (Canada Centre for Mineral and Energy Technology) and the Mining Association of Canada (MAC). The AETE program targeted the technology and tools available in the impact assessment process. Its mandate complemented AQUAMIN in the identification of affordable, effective tools to determine and characterize the impacts of mining operations on receiving waters.

The AETE program ran from 1994 to 1998. It was undertaken from a technical and economic (not regulatory) perspective and was based on the principle of sound science. The process did not make specific recommendations for application to regulations such as EEM (Environmental Effects Monitoring), but from the onset it was recognized that some of the technologies recommended by AETE may be adopted under a regulatory program.

The program mandate was outlined at an Initiation Meeting, November 23, 1993 to:

1. Evaluate environmental monitoring technologies that could be used by the mining industry and regulatory agencies in assessing the impacts of mine effluents on the aquatic environment in as cost-effective a manner as possible; and
2. Recommend specific methods or groups of methods that will permit accurate characterization of environmental impacts on the receiving waters in as cost-effective a manner as possible.

The scope of the program was similar to that of the AQUAMIN initiative and included:

1. Base metal mines, including copper, zinc, lead and nickel
2. Precious metals mines (excluding placer mining)
3. Uranium mines (excluding effects associated with radioactivity)
4. Ammonia, cyanide and salts

1.2.1 Program Organization

The program structure consisted of a Management Committee, a Technical Committee, and a Secretariat (Figure 1.1). Members of the AETE program are identified in Appendix A.

Management Committee

All participating organizations were members of the Management Committee, which set the direction and global priorities of the program. It also directed and reviewed the activities of the Technical Committee in accomplishing program objectives.

The Management Committee, in conjunction with the Technical Committee and the Secretariat, ensured that program objectives were realistically achievable, within the timeframe and resources allocated for their completion.

Technical Committee

The Technical Committee was responsible for implementing the program. It set work priorities and ensured completion of the work program within the financial and time limits of the program.

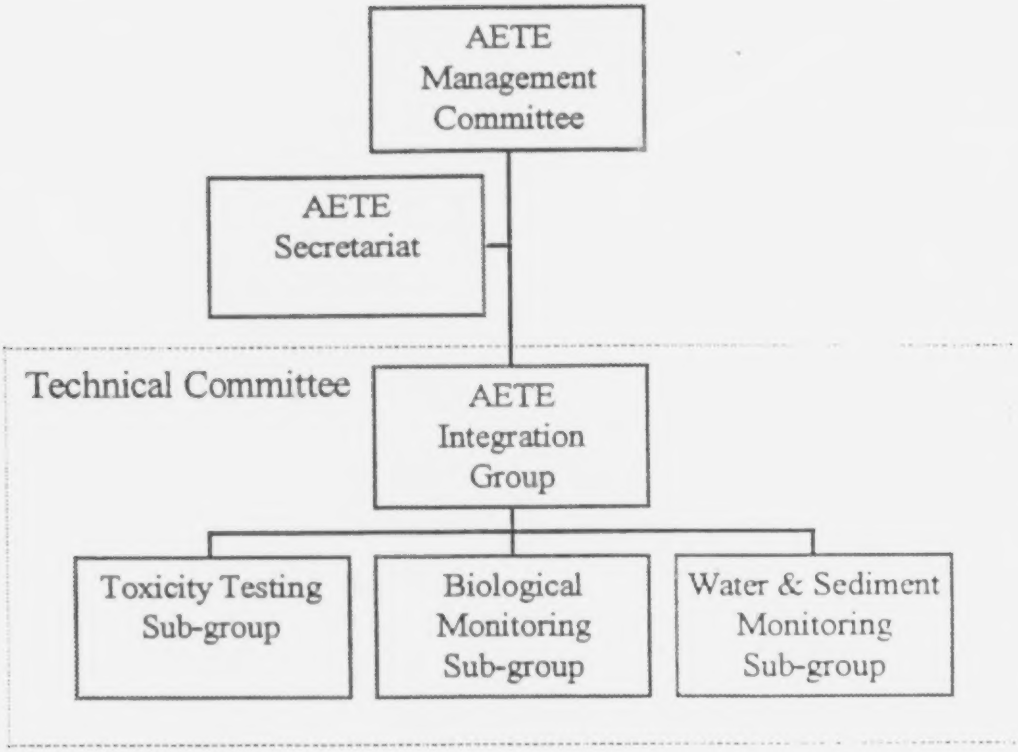

AETE Management Committee
AETE Secretariat
Technical Committee
AETE Integration Group
Toxicity Testing Sub-group
Biological Monitoring Sub-group
Water & Sediment Monitoring Sub-group

Figure 1.1 AETE Program Structure

The Technical Committee was originally divided into three sub-groups dealing with water and sediment monitoring; toxicity testing; and biological monitoring in receiving waters. In 1996, the three sub-groups merged into one large committee for more effective information sharing and to ensure integration of methods through the field evaluations conducted in 1996 and 1997. Task Force Groups composed of representatives of all stakeholders were formed to work on the various work elements.

An Integration Group was established to ensure coordination of the Technical Committee activities. The Integration Group consisted of approximately ten (10) members representing the various stakeholder groups. The Integration Group acted as a technical decision making body and provided a link between the Management Committee and the Technical Committee.

1.2.2 Participants

The AETE program included participants from several different sectors. The major groups along with their roles and responsibilities are outlined below. All participants provided technical expertise where appropriate.

Natural Resources Canada (NRCan)

Natural Resources Canada, through CANMET, lead and coordinated the program. CANMET provided $2.2 million of the $3.4 million budget, and the Secretariat for the program. CANMET chaired the Management Committee. The Geological Survey of Canada (part of NRCan) also participated on the Technical Committee.

Mining Association of Canada

The Mining Association of Canada (MAC) represents the mining industry in Canada and contributed $1.2 million toward the AETE budget. MAC members participated on both the Management and Technical Committees. As well, the Association provided pertinent in-house technical information from its members and access to field sites for the assessment of selected monitoring technologies.

Environment Canada

Environment Canada participated on the Management Committee and on the Technical Committee. The AQUAMIN representative from Environment Canada ensured communication between AQUAMIN (the federal regulatory initiative on aquatic effects assessment) and the AETE program.

Other Federal Departments

The Department of Fisheries and Oceans and Indian Affairs and Northern Development Canada participated on the Management Committee, and on the Technical Committee.

Provincial Governments

Provincial government representatives participated on the Management Committee and on the Technical Committee. The provincial governments involved were British Columbia, Manitoba, Saskatchewan, Ontario, Quebec (up to 1996), New Brunswick, Nova Scotia and Newfoundland.

1.3 AQUAMIN

The AQUAMIN (Aquatic Effects of Mining in Canada) program focused on the regulatory issues associated with the impacts of mining effluents. The objective of AQUAMIN was to examine the effectiveness of the MMLER by reviewing case studies on the environmental impacts of mining relative to the existing legislation. On the basis of this assessment, recommendations were formulated in three key areas:

1. amendments to the MMLERs,
2. design of a national EEM program for metal mining, and
3. information gaps and research needs.

The program began with the compilation of over 700 documents pertaining to the aquatic environmental effects of mines in Canada. Many of these were unpublished reports prepared by individual mining companies. The documents were reviewed and summarized into a comprehensive database that is now housed at Laurentian University, Sudbury, Ontario.

Detailed case studies for 18 sites were prepared by four working groups divided on the basis of mine location. The case studies reviewed whether an environmental effect was determined at a mine site and the nature and magnitude of the effect. The case studies identified sites where receiving environment conditions have improved over time as a consequence of improved effluent treatment and wastewater management. From the review it was not possible to evaluate with confidence the effectiveness of the concentration limits of MMLER parameters. However, it was concluded that the current MMLERs may not be sufficient to protect fish, fish habitat and the use of fisheries resources at all mine sites.

It was recommended that cyanide be added to the existing list of MMLER parameters (arsenic, copper, lead, nickel, radium-226, the lower pH limit, total suspended solids and zinc) measured in effluent. Other substances in effluent of potential concern (e.g. other metals, nitrogen compounds, thiosalts) will be addressed through site specific monitoring requirements for periodic effluent characterization.

A major observation in reviewing the 700 documents and 18 case studies was a lack of consistency in the monitoring studies including study objectives, approaches and methods used. Therefore, one of the core AQUAMIN recommendations was to develop a comprehensive EEM program for the mining industry sector. The conceptual approach for a mining EEM program, as proposed by AQUAMIN, is illustrated in Figure 1.2. The actual program may differ but will be designed to incorporate a consistent national framework that allows site specific modifications.

An important consideration for the proposed mining EEM program is that it includes a phased approach from site characterization to focused monitoring with increasing levels of detail. A substantial part of the resources and effort of the (former) AQUAMIN working group is now directed toward developing an EEM program for the mining sector. Development of the appropriate technical guidance is expected by the middle of 1999, with implementation of the legislation targeted for late 1999.

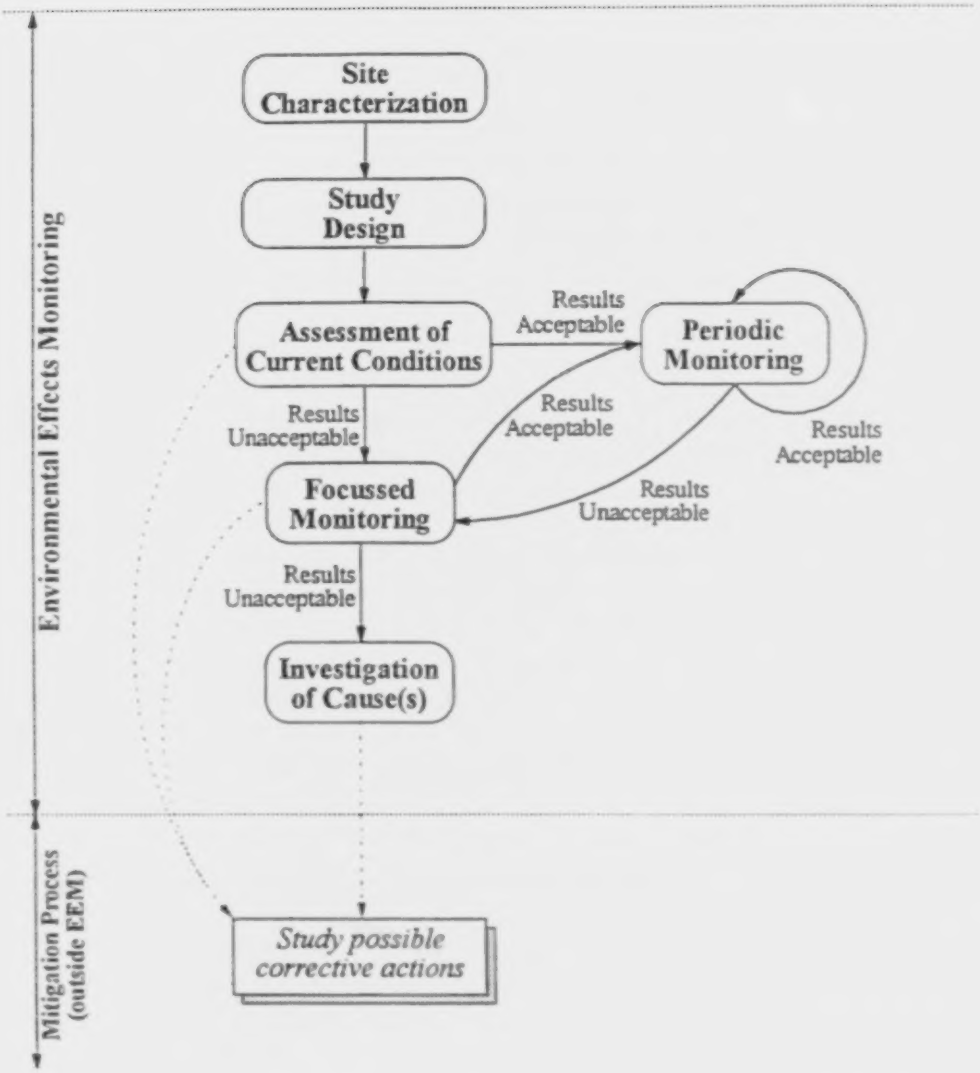

Environmental Effects Monitoring
Mitigation Process (outside EEM)
Site Characterization
Study Design
Assessment of Current Conditions
Results Acceptable
Periodic Monitoring
Results Unacceptable
Results Acceptable
Results Acceptable
Focussed Monitoring
Results Unacceptable
Results Unacceptable
Investigation of Cause(s)
Study possible corrective actions

Figure 1.2 Proposed Mining EEM Program Activities (from AQUAMIN Final Report, 1997)

1.4 Environmental Effects Monitoring (EEM)

The pulp and paper sector was first to be regulated under an EEM program in Canada and there are lessons to be learned for the mining industry. The pulp and paper EEM program is now a requirement under the *Fisheries Act*. It was realized that different Canadian pulp and paper mills previously conducted different environmental monitoring programs in response to government priorities and emerging environmental issues. AQUAMIN made a similar observation for mining across Canada. To achieve national uniformity in such studies, an EEM requirement was incorporated into the amended *Pulp and Paper Effluent Regulations* (PPER) under the *Fisheries Act*. The adequacy of existing effluent regulations is also being assessed by undertaking EEM studies at all locations where effluent is discharged to aquatic receiving environments.

The First Cycle EEM studies for pulp and paper were completed between 1994 and 1996. The experience and lessons from these studies were available to the AETE program and aspects of the fish survey are briefly reviewed in Chapter 6. The AETE Management Committee invited input from individuals with EEM experience in the pulp and paper program from both industry and government. This was particularly true toward the end of AETE.

Development of an EEM program is consistent with many objectives of the federal government at the national and international levels. Canada is recognized as one of the foremost producers of minerals and metals in the world. At the same time it is committed to achieving *Sustainable Development* (Minerals and Metals Policy of the Government of Canada 1996). There are many ambitious programs aimed at establishing environmental regulations and standards by international institutions such as the United Nations Environment Program (UNEP) and the Organization for Economic Cooperation and Development (OECD). As signatories to these programs, Canada has an obligation to manage the environmental effects of its own industries.

The primary objective of EEM for mining is to evaluate the effects of mining activity on the aquatic environment including fish, fish habitat and the use of fisheries resources (AQUAMIN, 1996). EEM requires a set of tools (monitoring techniques) that can provide the weight-of-evidence that ecological effects are occurring and that these effects can be attributed to discharges of mine-related wastes. The selection of appropriate tools for use in EEM is, however, a scientifically challenging endeavor. The rationale may originate from policy, but good science must be the foundation of all EEM components.

A mining EEM program is currently being designed by a multi-stakeholder group including Environment Canada and the Mining Association of Canada. The recommendations of useful, cost-effective monitoring tools by AETE will be considered in the design of the mining EEM program.

1.5 Specific Aspects of Mining in an EEM Framework

Some of the specific considerations of mining pertinent to environmental monitoring are identified and briefly described below.

- Location of mine sites often near headwaters

Many mine sites are located in remote areas near the headwaters of rivers or streams. Therefore, effluent flow can constitute a very significant portion or even a majority of the river flow downstream of the discharge point. The main consequence is that the available dilution and mixing is reduced and effects to the biota can be important. Also, since mines are often found at headwaters, it is difficult to find suitable reference sites and the downstream effects caused by industrial discharges are, therefore, harder to determine.

Similarly, headwater areas are often not suitable as fish habitat. This is due to stream size or gradient (i.e. very steep in the mountains). However, it is possible to see more sensitive fish species (ex. Brook trout, salmon, etc.) in headwater areas where mine sites are commonly located.

- Size and lifespan of mines

Many mines are small, both in area affected and releases, and short in lifespan (10-15 years). Many metal mines mainly obtain ore from underground with limited surface disturbance leaving only a tailings deposit site.

- Mining effluents contain naturally occurring elements (metals)

Most components of mining effluent are of inorganic nature. Metals are natural substances that cannot be destroyed, therefore they are persistent. They may have different forms or complex with organic matter, but remain natural substances. Most of the other compounds used in the mining industry are adsorbed on product concentrates and tailings or are degraded before being discharged, and are rarely identified as environmental problems except ammonia and cyanide (note that not all mines use cyanide as a reagent).

- The crucial role of local geology in setting background conditions at mine site

Mineralization of an area can often affect the natural water quality of streams near mine sites. Local mineralization conditions should take preference over generic water/sediment quality guidelines when assessing mine-related effects, since they are naturally occurring in nearby water and sediment.

- Bioavailability of metals in the downstream aquatic environment

The ecological effects of metals in the environment are largely determined by the bioavailability of metals and also the modifying factors that can change this availability. Discharges from mines often contain metals in insoluble particulate forms and even the effects of soluble metals can be mitigated by the beneficial effects of hardness, especially in lime treated effluents.

- General water management requirements at mine sites

Effluent discharge rates are often not consistent year round. In fact, many mines in the colder regions of Canada may not discharge at all during winter months or will discharge at minimal rates because of site specific issues. Reasons may include the need to protect overwintering fish where natural stream flow can be minimal during the winter, or to allow for natural degradation of cyanide complexes during warmer periods.

Conversely, in the spring discharge rates may be very high to relieve water inventories while stream flows are high. Thus, peak potential contaminant loading can be discharged when the available dilution is high. The retention time available in a mine's tailings pond also has an effect on cyanide breakdown.

At certain times of the year or at certain locations, there can be a need to minimize discharges. Such a circumstance could occur in late summer when stream flows are low and fish may be spawning in areas below a mine. To accommodate the above requirements, mines can have very large storage capacities for water that may or may not require treatment before discharge. Water management is therefore a very high priority in all operations.

- Variability in nature/volume of effluents from mines

Mines are not chemical process plants that can control the properties of ores and waste materials that must be processed or managed. The very nature of mineral deposits is the primary factor which controls contamination in waters before treatment and is reflected in subsequent effluent quality. Also, the acid generating potential in ores can vary from various mines and from within the same mine. Virtually every mine has its own distinct suite of parameters of concern in effluents along with individual levels of mitigating factors related to bioavailability and hardness.

1.6 Objectives of the Synthesis Report

The AETE technical program covered a four year time frame and included over 30 individual studies ranging from literature reviews to extensive cross-Canada field programs. This Synthesis Report provides a summary and overview of these activities as well as a chronology of the events. It does not provide results and detailed information from each study. The individual AETE reports listed in Table 2.1 should be consulted for further information.

This Synthesis Report provides the final recommendations for the monitoring tools evaluated by the AETE process. During the 4 year span of the AETE program, many reports were prepared by various authors or groups. Individual reports provided conclusions and recommendations based on their separate findings including technical evaluations and field studies. All this information was reviewed by the Technical Committee to identify the monitoring tools considered appropriate for the mining industry in Canada. Where there were discrepancies between a Technical Review and the findings of the AETE field surveys concerning a particular tool, greater weight was given to the field survey results when considering the final recommendations. **In the end, only the tools recommended in this Synthesis Report are endorsed by AETE, and these recommendations take precedence over previous individual reports prepared within the AETE program.**

Monitoring tools considered by the AETE program had to address at least one of the four following questions:

1) are contaminants getting into the system?

2) are contaminants bioavailable?

3) is there a measureable biological response? and,

4) are the contaminants in the system causing the observed response?

These practical questions formed the basis for much of the direction taken by the program. In recognition of their importance to AETE, much of the organization of this report is designed around these questions. Each of the questions is addressed separately in Chapters 4-7, along with a discussion of the relevant and appropriate monitoring tools. It is recognized that certain tools could be applied to more than one question. In these situations, cross-referencing between chapters is provided.

The reader must remember that this report is not a guidance document or "how to" manual for conducting EEM studies. The tools recommended for use by the AETE program are considered suitable for routine monitoring programs. Several tools were not considered suitable for routine monitoring but are recognized to have potential application for detailed site specific studies. Further discussion on tool selection and their application in routine monitoring is provided in Section 2.2.

The AETE program was an intensive process involving many people with varied backgrounds, perspectives and interests. As such, a rich mosaic of information, knowledge and opinions was available. This report is meant to reflect the tremendous effort exercised by the AETE participants, the care in developing recommendations by consensus, and finally, to capture the spirit and philosophy of both the program and its members.

2. AETE TECHNICAL PROGRAM

2.1 Overview

The AETE program consisted of three main technical areas:

1) acute and sublethal toxicity testing methods;
2) biological monitoring methods in receiving waters; and
3) water and sediment monitoring methods.

It was the intent of the program to identify a suite of methods which have demonstrated environmental and economic effectiveness in monitoring the receiving waters of mining effluents. In the early stage of the program, a preliminary list of candidate tools was developed at AETE meetings (Appendix B). Potential monitoring methods were screened according to the following two primary criteria: 1) the methods have been successfully applied in the field (sound science literature base); and 2) the cost of obtaining the samples from the field and analyzing in the laboratory was known. This exercise identified methods suitable for field evaluation and a number of "screened-out" tools not considered further. For example, metal levels in benthos, zooplankton and bacteria. Other possible tools, such as sediment toxicity testing, were added later in the program.

Where a technique was considered promising but did not meet the above criteria, the tool was typically reviewed through a technical evaluation by an individual or team with experience with that monitoring technique. As a rule, monitoring tools in the early research stage were not considered further. Evaluations of a particular monitoring tool included one or more of the following:

- literature review and opinion;
- laboratory testing; and
- field evaluation.

The AETE program also addressed some issues and techniques applicable to mining but they were not evaluated as routine monitoring tools. These included Toxicity Identification Evaluation (TIEs) procedures, the influence of highly mineralized water (HMW) on effluent toxicity and application of the sediment quality triad for data interpretation. They did not address a particular hypothesis or guiding question. These topics are discussed in this report but they are not considered monitoring tools, therefore, there is no recommendation concerning their suitability in a routine monitoring program.

The AETE sponsored over 30 separate studies that ranged from literature reviews of single tools to large field programs involving multiple sites across Canada and numerous tools. The studies produced from the technical evaluations are listed in Table 2.2. This list provides insight to the range of issues addressed and completed by the AETE program. The goals of each of the three main technical areas are outlined below.

2.1.1 Toxicity Testing Program

The Toxicity Testing program had two goals. The first was to evaluate alternatives to the rainbow trout and *Daphnia magna* acute lethality tests, which are widely used to evaluate the acute effects of effluents. The purpose was to determine what tests (if any) can provide accurate information on effluent acute toxicity, but at lower cost and more quickly. The second goal was to evaluate sublethal toxicity test methods for assessing sublethal effluent impacts at least cost. An important distinction was that sublethal toxicity results were also to be used to predict downstream biological impacts.

Acute Toxicity Testing

The rationale for the evaluation of acute toxicity alternatives is that the current methods (and particularly trout) are relatively slow and expensive.

In 1997, the total cost of a trout and *Daphnia* bioassay to a mining operation, including laboratory fees and the cost required for sample collection and shipping, is in the order of $1,000.00. The costs for sampling one effluent stream four times a year are not high. However, such costs increase substantially when weekly or monthly samples must be taken from a number of effluent streams or receiving environments.

Five quick microtests were identified as potential alternatives to the rainbow trout test and were evaluated in the laboratory using a variety of mine effluents. The results obtained from all toxicity testing were analyzed, and recommendations were made on the feasibility of the alternatives.

Sublethal Toxicity Testing

Sublethal toxicity testing has been less commonly used than acute testing in regulatory programs, but its use in the future is anticipated to increase as more sensitive methods are being considered. In addition, AETE was committed to examine the use of sublethal toxicity test results for predicting downstream biological effects. This particular consideration provides a valuable contribution to the science of environmental monitoring.

A critical review was conducted to provide information on the broad application of routine sublethal toxicity testing for the Canadian mining industry. Candidate sublethal toxicity test methods were assessed in terms of cost, speed, reproducibility, and sensitivity.

A list of 13 sublethal tests was originally developed for consideration. Of these, 9 tests were shortlisted for laboratory evaluation. The performance of each shortlisted test method was compared by testing of representative mining effluents through three rounds of testing. Four sublethal tests were eventually recommended by AETE. The possible amelioration effect of receiving water quality on toxicity test results was examined by collecting upstream water at a mine for use as dilution water.

Table 2.1 List of AETE Projects (* internal AETE reports)

1.0	TOXICITY TESTING PROGRAM	
	1.1.1	Evaluation of Standard Acute Toxicity Tests for Selected Mine Effluents (Conduction of Rainbow Trout, *Daphnia magna* and *Daphnia magna* IQ Tests for Selected Mine Effluents) * Results published in #1.1.4
	1.1.2	Evaluation of Alternative Acute Toxicity Tests for Selected Mine Effluents (Conduction of Microtox, Rotoxkit F, Thamnotoxkit F and Toxichromotest Tests) * Results published in #1.1.4
	1.1.3	Chemical Analysis of Selected Mine Effluents (Acute Toxicity Project) * Results published in #1.1.4
	1.1.4	Comparison of Results from Alternative Acute Toxicity Tests with Rainbow Trout for Selected Mine Effluents. April, 1996
	1.2.1	Review of Methods for Sublethal Aquatic Toxicity Tests Relevant to the Canadian Metal-Mining Industry. April, 1997
	1.2.2	Laboratory Screening of Sublethal Toxicity Tests for Selected Mine Effluents. January, 1997
	1.2.3	Chemical Analysis of Selected Mine Effluents (Sublethal toxicity project) *
	1.2.4	Toxicity Assessment of Highly Mineralized Water from Potential Mine Sites. July, 1997
	1.2.5	Evaluation of Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) Application to the Canadian Mining Industry. November, 1998
	4.1.2a	Toxicity Assessment of Mining Effluents Using Up-stream or Reference Site Waters and Test Organism Acclimation Techniques. July, 1997
2.0	BIOLOGICAL MONITORING PROGRAM	
	2.1.1	Review of Artificial Substrates for Benthos Sample Collection. June, 1995
	2.1.2	Optimization of Field and Laboratory Methods for Benthic Invertebrate Biomonitoring. January, 1997
	2.1.3	Technical Evaluation of Methods for Benthic Invertebrate Data Analysis and Interpretation. June, 1997
	2.1.3a	Review of Potentially Applicable Approaches to Benthic Invertebrate Data Analysis and Interpretation. March, 1999
	2.1.4	Quality Assurance Program for Assessing Mine-related Effects Using Benthic Invertebrate Communities. February, 1999
	2.1.5	Determining Mining Related Impacts Utilizing Macroinvertebrate Population Fitness Parameters. February, 1999
	2.2.1	Technical Evaluation of Metallothionien as a Biomarker for the Mining Industry. February, 1997
	2.2.2	Technical Evaluation of Histopathology as an Environmental Monitoring Tool for the Mining Industry in Canada. June, 1997
	2.2.3	Technical Evaluation of Fish Methods in Environmental Monitoring for the Mining Industry in Canada. February, 1999
	2.3.1	Technical Evaluation of Mollusc as a Biomonitoring Tool for the Canadian Mining Industry. March, 1997
	2.3.2	Technical Evaluation of Monitoring Methods Using Macrophytes, Phytoplankton and Periphyton to Assess the Impacts of Mine Effluents on the Aquatic Environment. November, 1997
3.0	WATER AND SEDIMENT MONITORING PROGRAM	
	3.1.1	Technical Evaluation on Sample Collection, Handling, Analysis and Interpretation for Trace Level Contamination in Water. January, 1999
	3.1.2	Technical Evaluation on Water Quality and Biological Effects. May, 1997
	3.1.3	Cost-effective Protocols for the Collection, Filtration and Preservation of Surface Waters for Detection of Metals and Metalloids at ppb ($\mu g\ L^{-1}$) and ppt ($ng\ L^{-1}$). Phase I: Evaluation of Bottle Type, Bottle Cleaning, Filter and Preservation Technique. April, 1998
	3.2.1	Literature Review on the Techniques of Sonar Profiling and Grid Sampling, Using a Grab Sampler for the Identification and Mapping of Lake Sediment Facies for Environmental Effects Monitoring. August, 1995
	3.2.2a	Assessing Aquatic Ecosystems Using Pore Waters and Sediment Chemistry. December, 1998
4.0	INTEGRATED STUDIES	
	4.1.1	1995 Field Evaluation of Aquatic Effects Monitoring Methods – Pilot Study (2 volumes). September, 1996.
	4.1.2	1996 Field Evaluation of Aquatic Effects Monitoring Methods (9 separate reports). December, 1996
	4.1.2a	Toxicity Assessment of Mining Effluents Using Up-stream or Reference Site Waters and Test Organism Acclimation Techniques. July, 1997
	4.1.3	1997 Field Evaluation of Aquatic Effects Monitoring Methods (5 separate reports). March, 1999
	4.1.4	AETE Program Synthesis Report of Selected Technologies for Cost-Effective Environmental Monitoring of Mine Effluent Impacts in Canada. March, 1999

2.1.2 Biological Monitoring Program

The mandate of the Biological Monitoring Sub-Committee was to conduct a field evaluation of methods for assessing the biological impacts of mine effluents on receiving waters. At present, a large (and growing) number of methods are being used with little standardization from site to site. The objective was to determine which tools, or suites of tools, can adequately characterize effluent impacts on receiving waters, in a cost-effective manner. Standardization would result in improved and less subjective characterization of receiving water impacts, and an ability to more readily compare impacts among sites.

A major obstacle to standardization of biological monitoring techniques is the large number of potential techniques available. Relevant classes of organisms include fish, benthos, zooplankton, phytoplankton, macrophytes and bacteria. Within each class of organism there exist intracellular, tissue, organism, population and community level effects that can be measured (see also Section 6.0 for further discussion).

Even within these subsets, there is tremendous variability in the methods available, and in their interpretation. This range of choices is necessary and appropriate in research studies on aquatic ecosystems; however, it is inappropriate, expensive and confusing for routine monitoring programs.

2.1.3 Water and Sediment Program

The mandate of the Water and Sediment Monitoring Sub-Committee was to evaluate methods of assessing the impacts of mine effluents on receiving waters and their underlying sediments. There has been little standardization to date for sediment and for filtered water ("dissolved") samples collected for impact assessment. Many of the commonly used techniques for sediments are inappropriate for the collection of environmentally useful data. Similarly, different filter types, even of the same pore size, may give quite different results for the same water or effluent sample. The ability to measure effects particularly in water, is also influenced by analytical detection limits. Therefore, analytical methods available for chemical parameters of interest to the mining industry were reviewed.

2.2 Selection of Monitoring Tools

To evaluate the effects of mining effluents on aquatic environments, tools are required that can reliably determine if impacts have occurred. The selected tools must be responsive to the effects of mining and reflect ecosystem characteristics that are relevant. It is important to note that the AETE program defined "effect" as:

"A measurable difference in an environmental variable (chemical, physical or biological) between a point downstream in the receiving environment and an adequate reference point (either spatial or temporal)."

To construct a burden of evidence for effects, selected tools should be able to answer one or more of the following questions:

(1) are contaminants getting into the system?

(2) are contaminants bioavailable?

(3) is there a measurable biological response? and,

(4) are the contaminants in the system causing the observed response?

These four guidance questions provided useful direction to the AETE program for selecting and evaluating tools. The questions reflect the philosophy of the AETE members and form the organizational basis for a significant portion (Chapters 4-7) of this report.

Of the four guiding questions, measurable biological or ecological responses are probably the most critical for the evaluation of effects. For example, the presence of elevated metal levels and demonstrated biological availability may not be interesting unless there was also an observed biological response. In contrast, if there was an observed ecological response, but there were no elevated metal levels in the environment, and no apparent biological availability of metals, there would still be interest in determining the underlying cause of the biological change. As a consequence, it is important to ensure that the methods selected for characterizing the ecological responses are reliable for demonstrating effects when they occur.

To minimize the detection of effects that are considered trivial, programs should select monitoring methods that have relevance to the features that are being protected by the underlying legislation (Cairns *et al.*, 1993). In Canada, environmental monitoring is driven largely by the *Fisheries Act* which has a goal of maintaining the productive potential of fish and fish habitat. As a consequence, assessment programs in Canada should monitor aspects of fish populations and communities, as well as those attributes (e.g., chemical and physical characteristics of habitat as well as benthic community composition) that provide direct linkages to the fishery resource.

The AETE program considered well over 100 potential monitoring tools through initial screening, technical evaluations and field trials. Tools were evaluated using several criteria including ability to answer one or more of the four guidance questions, demonstrated performance in the AETE program, cost, practicality and availability of standardized protocols.

The tools and methods that are recommended should be suitable for use in a routine monitoring program by today's standards. **A routine monitoring program is one designed to establish existing conditions and to determine if there are measureable effects (see definition of "effect" above).** A routine monitoring program must be properly designed to achieve these objectives, but it is not expected to fully delineate the spatial extent or magnitude of effects, identify all ecosystem components potentially affected, or to identify mechanisms or cause of effect. In some cases, tools were not recommended by AETE for routine monitoring but may be appropriate for more detailed site-specific applications. Only tools tested within the context of AETE were recommended, and may not apply at all sites. Specifically, it should also be noted that AETE did not evaluate tools for marine environments.

Hypotheses Testing

In early 1996, the Technical Committee formulated a series of formal hypotheses to be tested through field programs conducted at a number of mining discharge locations in 1997. These hypotheses were based on the four questions cited previously. The hypotheses helped clarify program objectives and identify areas where AETE could make a valuable scientific contribution. In addition, monitoring tools were, in part, selected on their ability to address the hypotheses (see Sections 3.3, 3.4.2 and Table 3.2 for further details).

Cause-Effect Linkages

A significant component of the AETE program was to examine the relationships between various ecosystem components (e.g. sediment chemistry and benthos). This is reflected in five of the hypotheses (Integration of Tools) that attempt to better identify cause-effect linkages. To confirm a linkage between the observed ecological response and metal-mine effluent (are contaminants in the system causing the response?), a weight-of-evidence approach can be taken. The weight-of-evidence can be constructed, in part, with an appropriate study design that compares either spatial and/or temporal reference data (Taylor, 1997).

The AETE study designs and statistical methods for hypothesis testing are described in Section 3.3 and 3.4. Chapter 7 also outlines some of the methods used to identify relationships between different ecosystem variables. These include correlation analysis, the sediment quality triad and the weight-of-evidence approach.

Table 2.2 Overview of Guiding Questions, Toolboxes and Tools Considered for the AETE Program

Question	Toolbox	Tool	
Are contaminants getting into the system?	Effluent Chemistry	1.1	Chemical parameters
	Water Chemistry	2.1	Total metal concentrations
			Dissolved metal concentrations
	Sediment Chemistry	3.1	Substrate mapping
		3.2	Surface sediment collection
		3.3	Sediment cores
		3.4	Total metal concentrations
		3.5	Partial metal concentrations
		3.6	Porewater
	Effluent Acute Toxicity	4.1	*Daphnia* IQ test
		4.2	Microtox acute test
		4.3	Rototoxkit F test
		4.4	Thamnotoxkit F
		4.5	Toxichromotest
		4.6	Rainbow trout
		4.7	*Daphnia magna*
Are contaminants bioavailable?	Aquatic Plants	5.1	Metal concentrations
	Molluscs	6.1	Tissue metal concentrations
	Fish Tissues	7.1	Organ tissue metal levels
		7.2	Tissue metallothionein levels
Is there a measurable response?	Effluent Sublethal Toxicity	8.1	Fathead minnow
		8.2	*Cenodaphnia dubia*
		8.3	*Selenastrum capricornutum*
		8.4	*Lemna minor*
		8.5	Phytoplankton multi-species test
		8.6	Microtox chronic

Table 2.2 Overview of Guiding Questions, Toolboxes and Tools Considered for the AETE Program

Question	Toolbox	Tool	
		8.7	Mutatox chronic
		8.8	Nematode survival
		8.9	Rainbow trout embryo
	Sediment Toxicity	*9.1*	*Chironomus riparius*
		9.2	*Hyalella azteca*
		9.3	*Tubifex tubifex*
		9.4	Microtox solid phase
	Aquatic Plants	10.1	Phytoplankton community structure
		10.2	Periphyton community structure
		10.3	Macrophyte community structure
		10.4	Biochemical indicators
	Benthic Invertebrates	11.1	Mollusc growth
		11.2	Fitness parameters
		11.3	Community composition
		11.4	Artificial substrates
		11.5	Rapid assessment procedures
	Fish Survey	12.1	Biochemical indicators
		12.2	Histopathology
		12.3	Fish organ size
		12.4	Fish growth
		12.5	Abundance indicators
		12.6	Community indicators
Are contaminants causing this response?	Pairwise comparisons of the above toolboxes	13.1	Chemistry vs biology correlations
		13.2	Toxicity vs biology
		13.3	Chemistry vs toxicity

3. FIELD EVALUATION OF AQUATIC MONITORING TOOLS

3.1 Introduction and Overview

A significant part of the AETE mandate was to undertake field evaluations of aquatic monitoring technologies that could be used to assess impacts of mines on the aquatic environment. The focus of the field program was on robustness, cost and suitability of monitoring methods for the mining industry. The field programs were not conducted for the purpose of determining the effects of a specific mine operation on receiving waters. Furthermore, AETE chose a range of operating conditions and site characteristics that did not necessarily reflect ideal experimental sites but it was the intent to test tools at real mining sites. Therefore, these confounding factors were sometimes present and selection of reference areas was challenging.

The AETE field program encompassed three years and included the following studies:

1995: Pilot Study: Pilot field and laboratory evaluation at Val d'Or, Quebec

1996: Phase I: Preliminary evaluation of 7 candidate sites with recommendations and proposed study design for subsequent detailed evaluation at four sites. The seven sites were:

- Myra Falls, British Columbia, (Westmin Resources)
- Sullivan, British Columbia (Cominco)
- Lupin, Northwest Territories (Echo Bay)
- Levack/Onaping, Ontario (INCO and Falconbridge)
- Dome Mine, Ontario (Placer Dome)
- Gaspé Division, Quebec (Noranda)
- Heath Steele Division, New Brunswick (Noranda)

1997: Phase II: Detailed field evaluation and hypothesis testing at four sites:

- Myra Falls, British Columbia, (Westmin Resources)
- Dome Mine, Ontario (Placer Dome)
- Heath Steele Division, New Brunswick (Noranda)
- Mattabi Mine, Ontario (Noranda)

Note: the Mattabi Mine was not considered in 1996, but there was extensive background information that made it suitable for detailed study in 1997.

1998: Phase III: Data interpretation and comparative assessment of the monitoring methods

Site locations for each of the field programs are shown in Figure 3.1. The following sections provide an overview of the scope and objectives of the field surveys. The actual results or recommendations from these programs are integrated under the various monitoring tools in the remainder of this document.

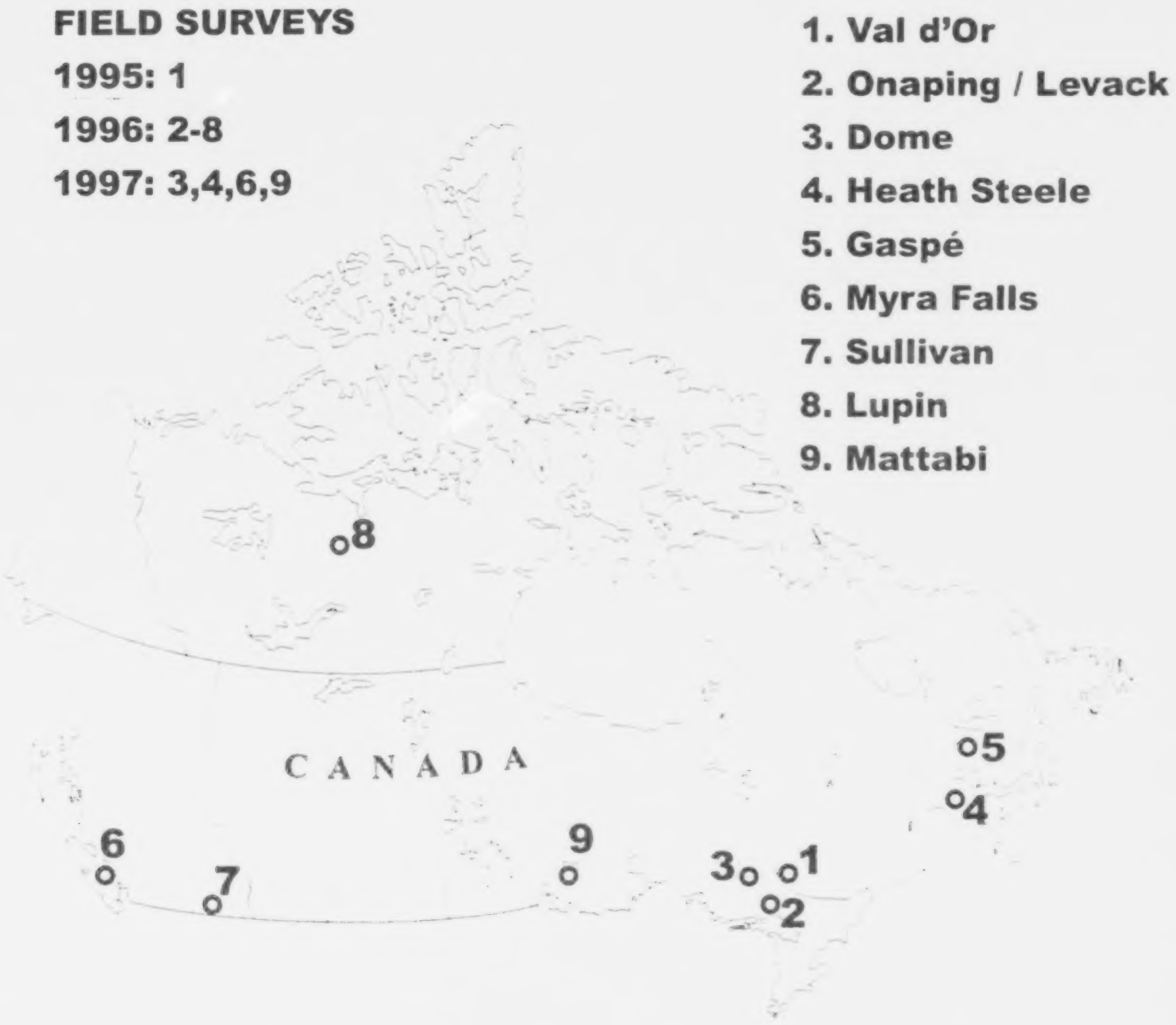

FIELD SURVEYS
1995: 1
1996: 2-8
1997: 3,4,6,9
1. Val d'Or
2. Onaping / Levack
3. Dome
4. Heath Steele
5. Gaspé
6. Myra Falls
7. Sullivan
8. Lupin
9. Mattabi
CANADA
8
6
7
9
3
1
2
5
4

Figure 3.1 Location of mine sites for AETE field evaluation.

FILE 98187_01

3.2 1995 Field Program

The pilot study was in the Val-d'Or region of Quebec, approximately 40 km east of Rouyn-Noranda (BEAK, 1996). It involved assessments in the receiving environments of Mine Doyon, Complexe Bousquet and Mine Dumagami. All three mines are primarily gold mining operations.

The AETE Technical Committee selected 10 monitoring tools for evaluation at the Val-d'Or pilot site which included:

1. Comparison of surficial sediment mapping techniques;
2. Comparison of the effectiveness of coring devices to quantify pre-operational metal levels;
3. Evaluation of the cost-effectiveness of using the lowest method detection limits achievable for water and sediment chemistry analyses;
4. Comparison of analytical methods for assessing sediment quality to predict biological effects by measuring either total metals in sediments by full extraction or those readily extractable (assumed to be more biologically available) by partial digestion;
5. Assessment of three sediment toxicity methods for their ability to predict biological effects from metal contamination of sediments;
6. Comparison of artificial substrates versus grab samples for benthos;
7. Evaluation of the cost-effectiveness and sensitivity of benthic invertebrate processing methods (sieve sizes, level of taxonomy, number of replicates) to delineate and monitor mining-related impacts;
8. Assessment of the effects of mining discharges on fish communities by evaluating the four main response characteristics; age structure, growth, energy storage and reproduction;
9. Evaluation of metal accumulation in various fish tissues (gill filament, kidney, liver, flesh, viscera); and
10. Comparison of metal levels in tissues to metallothionein levels and histopathology.

It should be noted that the 1995 field program was designed and implemented before development of the four guidance questions and thirteen hypotheses. The findings of the 1995 program were used in the selection of tools for further consideration.

3.3 1996 Field Program

Upon completion of the Pilot Study, the AETE field program entered Phase I: Preliminary field evaluations of seven candidate mine sites, selection of four sites for further work and preparation of study design for detailed field evaluations. The 1996 program was a preliminary reconnaissance of candidate sites and not for detailed evaluation of individual tools.

Phase I was undertaken in 1996 by a consortium of three consulting firms (EVS Consulting, ESG International, Jacques Whitford Environmental Ltd.) to provide a depth of technical expertise and simultaneously sample seven mine sites across Canada separated by several thousand kilometers. The fundamental steps carried out in Phase I included the following:

i) Review of background site-specific information;

ii) Preliminary field surveys: these surveys included habitat characterization, water and sediment collection, benthos and fisheries;

iii) Effluent collection for toxicity testing;

iv) Evaluation of site data and recommendations for detailed field evaluation; and

v) Development of detailed study design to test AETE hypotheses.

The project produced seven individual site reports, as well as a Recommendations Report and a Study Design Report. Highlights of the site selection process and recommendations are provided in this Chapter. For details on individual study results, the interested reader should refer to the original reports as follows:

Myra Falls Mine Site, 1996 Final Report. EVS. 1996a
Lupin Mine Site, 1996 Final Report. EVS. 1996b
Sullivan Mine Site, 1996 Final Report. EVS. 1996c
Levack/Onaping Site, 1996 Final Report. ESP. 1996a
Dome Mine Site, 1996 Final Report. ESP. 1996b
Heath Steele Mine Site, 1996 Final Report. JWEL. 1996a
Gaspé Mine Site, 1996 Final Report. JWEL. 1996b
1996 Survey: Recommendations for 1997 Sites. EVS/ESP/JWEL. 1997a
1997 Study Design Final Report: EVS/ESP/JWEL. 1997b

The general criteria provided by AETE to narrow the seven sites down to four or five for detailed evaluation were:

- Presence of a well defined gradient of effect (water, sediment, biological)
- Availability of adequate reference stations
- Suitability of site to test hypotheses

Site Evaluation Criteria

The information and data collected at each site were evaluated against a set of criteria to determine the suitability of the site for further testing. During the 1996 study, more detailed criteria were developed by the consulting consortium, and the AETE Technical Committee (Table 3.1), to choose sites for the 1997 field program.

Table 3.1 Summary of Criteria Used to Evaluate Candidate Sites in 1996

1. Availability of useful historical data (maximum 10 points)
   - 1.1 Effluent characterization
   - 1.2 Water chemistry
   - 1.3 Sediment chemistry
   - 1.4 Benthos
   - 1.5 Fisheries
     - 1.5.1 Population
     - 1.5.2 Tissues
2. Study Area (maximum 35 points)
   - 2.1 Site Access
     - 2.1.1 Is this site accessible by road?
     - 2.1.2 Is the reference area accessible by boat or road?
     - 2.1.3 Is the exposure area accessible by boat or road?
   - 2.2 Are multiple reference and exposure areas available?
   - 2.3 Are there "no" confounding point and non-point source discharges?
3. Effluent/Sublethal toxicity (maximum 30 points)
   - 3.1 Is effluent available year round?
   - 3.2 Does the effluent clearly exhibit chronic toxicity?
     - 3.2.1 *Ceriodaphnia dubia*
     - 3.2.2 Fathead minnow
     - 3.2.3 *Selenastrum capricornutum*
     - 3.2.4 *Lemna minor*
     - 3.2.5 Rainbow trout embryo test
4. Habitat
   Are habitats similar between Reference and Exposure areas? (maximum 10 points)
   - 4.1 Substrate
   - 4.2 Water depth
   - 4.3 Water velocity
5. Water Chemistry
   Are water chemistry concentrations statistically greater in Exposure area relative to Reference area? (maximum 15 points)
   - 5.1 Minimum of two general water chemistry parameters (e.g. sulphate, conductivity)
   - 5.2 Minimum of two metals (dissolved or total)
6. Sediments (maximum 20 points)
   - 6.1 Are representative depositional areas available?
   - 6.2 Are concentrations of at least two metals in sediments greater in Exposure vs. Reference area?
7. Benthos (maximum 15 points) Is there a significant difference between the Reference and Exposure areas?
   - 7.1 Total density
   - 7.2 Total species richness
   - 7.3 Richness of sensitive species (e.g. mayflies)
8. Fisheries (maximum 35 points)
   - 8.1 Community
     - 8.1.1 Are suitable sentinel species available in Reference and Exposure areas?
     - 8.1.2 Are sentinel species abundant (reasonable CPUE)?
     - 8.1.3 Are fish community differences apparent which can be linked to effluent?
   - 8.2 Fish tissue and histopathology
     - 8.2.1 Is there a difference in metallothionein levels between Reference and Exposure fish?
     - 8.2.2 Is there a difference in tissue metal levels between Reference and Exposure fish?
     - 8.2.3 Are there obvious differences in fish health between Reference and Exposure area fish?
   - 8.3 Do barriers to fish migration exist between Reference and Exposure areas?

The criteria listed in Table 3.1 were integral to the site evaluation process and also provide an indication of the study elements that were examined in Phase I. It is not practical to provide the results of each study component for each of the seven candidate sites in this Synthesis Report. Therefore, presentation of the evaluation criteria gives some insight into the scope of work that was undertaken during the 1996 field program. In Table 3.1, the relative weighting or importance of each criterion is also indicated by the maximum possible score for that particular element of the evaluation process.

To be as objective as possible, a numerical score was assigned to each criterion. The total score for each site (maximum score available was 170 points) was summed. Individual scores for each criterion at each mine site are provided in the report; *1996 Survey: Recommendations for 1997 Sites*. (EVS/ESP/JWEL, 1997b). The final scores as a percent of total for each site are summarized below in Table 3.3.

Hypothesis Testing

The second key consideration for selection of study sites for more detailed evaluation was to ensure they were suitable for hypotheses testing. The development and continual referral to a series of hypotheses that could be tested by the appropriate monitoring tools was an important core component of the AETE program. The hypotheses were initially developed by the AETE Technical Committee to clarify the purpose of program elements. These hypotheses were refined during 1996/97 and were formulated around the basic four AETE guidance questions.

The final hypotheses are listed in Table 3.2. The evaluation of integration methods was considered a priority within AETE, and hypotheses 9-13 are directed at examining relationships among study components. The methods used for hypothesis testing in Phase II and III are described in Section 3.4.2.

Table 3.2 Hypotheses for Testing in 1997 Field Evaluation

Sediment Toxicity:	
H_1:	*The strength of the relationships between sediment toxicity responses and any exposure indicator is not influenced by the use of different sediment toxicity tests or combinations of toxicity tests.*
Biological Monitoring - Fish:	
Metals in fish tissues (bioavailability of metals):	
H_2:	*There is no difference in metal concentrations observed in fish liver, kidney, gills, muscle or viscera (or whole fish).*
Metallothionein in fish tissues:	
H_3:	*There is no difference in metallothionein concentrations observed in fish liver, kidney, gills or viscera (or whole fish)*
Metals vs. metallothionein in fish tissues:	
H_4:	*The choice of metallothionein concentration vs. metal concentrations in fish tissues does not influence the ability to detect environmental exposure in fish to metals.*
Fish - CPUE:	
H_5:	*There is no environmental effect in observed CPUE (catch per unit effort) of fish.*
Fish or Benthic - Community:	
H_6:	*There is no environmental effect in observed fish or benthic community structure.*
Fish - Growth:	
H_7:	*There is no environmental effect in observed fish growth.*
Fish - Organ/fish size:	
H_8:	*There is no environmental effect in observed organ size (or fish size).*
Integration of tools:	
Relationship between water quality and biological responses:	
H_9:	*The strength of the relationship between biological variables and metal chemistry in water is not influenced by the choice of total vs. dissolved analysis of metals concentrations.*
Relationship between sediment chemistry and biological responses:	
H_{10}:	*The strength of the relationship between biological variables and sediment characteristics is not influenced by the analysis of total metals in sediments vs. either metals associated with iron and manganese oxyhydroxides or with acid volatile sulphides.*
Relationship between sediment toxicity and benthic invertebrate:	
H_{11}:	*The strength of the relationship between sediment toxicity responses and in situ benthic macro-invertebrate community characteristics is not influenced by the use of different sediment toxicity tests, or combinations of toxicity tests.*
Metals or metallothionein vs. chemistry (receiving water & sediment):	
H_{12}:	*The strength of the relationship between the concentration of metals in the environment (water and sediment chemistry) and metal concentration in fish tissues is not different from the relationship between metal concentration in the environment and metallothionein concentration in fish tissues.*
Chronic Toxicity - Linkage with Fish and Benthos monitoring results:	
H_{13}:	*The suite of sublethal toxicity tests cannot predict environmental effects to resident fish performance indicators or benthic macro-invertebrate community structure.*

The consulting teams involved in each of the mine studies considered the results of their surveys and determined which specific hypotheses could be tested at each site. For each site it was determined whether each hypothesis could be fully addressed, partially addressed or not addressed. The results of this evaluation along with the criteria scores for each of the candidate sites are summarized in Table 3.3.

Based on the site scores and consideration of hypotheses, four mine sites were chosen for further detailed evaluation in 1997: Dome, Heath Steele, Lupin and Myra Falls.

- The Dome Mine site in Ontario was clearly the primary candidate for further study in 1997
- The Gaspé and Heath Steele sites were similar, therefore only one was considered further; sublethal toxicity was more evident at Heath Steele
- Lupin had the next highest score and was recommended
- Myra Falls and Onaping/Levack had similar scores but more hypotheses could be addressed at Myra Falls, therefore, it was recommended for further study

Table 3.3 Summary of Criteria Scores for Sites Studied in 1996

Mine Site	Percentage Score	Hypotheses Fully Addressed	Hypotheses Partially Addressed	Hypotheses not Addressed
Dome	78%	10	2	1
Gaspé	69%	3	6	4
Heath Steele	67%	3	6	4
Lupin	63%	10	3	0
Myra Falls	61%	6	4	3
Onaping/Levack	61%	3	6	4
Sullivan	57%	2	8	3

Recommended Study Designs for 1997

The study teams involved in the 1996 site surveys developed preliminary study designs that could be used for hypotheses testing during the 1997 field program. These were subsequently modified for application in 1997 and are described in Section 3.4.

Each type of hypothesis required a different statistical model to generate the appropriate data for analysis and interpretation. There are essentially two basic study designs for field surveys: a) Control-Impact (CI) design with samples from a Reference and Exposure area, and b) gradient design where samples are collected along a gradient of environmental conditions.

Recommended samples sizes were developed for the sites based on statistical power and required effect size. For CI designs, it was determined that sampling should be adequate to detect differences of one standard deviation (SD). For gradient designs the desired correlation between variables should be at least ≥ 0.50 or 0.60. It was estimated that 20-25 field samples per site represented a reasonable trade-off between feasibility, cost, statistical power and robustness.

3.4 1997 Field Program

3.4.1 Overview

In 1997, the AETE program continued with the last two Phases of the Field Evaluation:

- Phase II: Detailed field and laboratory evaluation and hypotheses testing at four sites
- Phase III: Data interpretation and comparative assessment of the monitoring methods.

The scope of Phase II is presented below in this chapter while the results of Phase II and III are implicit in the tool recommendations provided throughout this report.

In 1997, detailed field studies were conducted at the following sites:

1. Heath Steele, Miramichi, New Brunswick (BEAK 1998a)
2. Dome Mine, Timmins, Ontario (BEAK 1998b)
3. Myra Falls, Vancouver Island, British Columbia (BEAK/Golder 1998a)
4. Mattabi Mines, Ignace, Ontario (BEAK/Golder 1998b)

It should be noted that the Lupin mine in the Northwest Territories was originally recommended for further testing based on the 1996 field survey (see previous Section 3.3). However, a reconnaissance survey early in 1997 determined there was a lack of a well defined gradient of metal levels surrounding the mine. Therefore, the AETE Technical Committee substituted the Mattabi mine in Ontario. Background information and access to the Mattabi site were readily provided by the mine owners, Noranda Mining and Exploration Inc.

The 1997 field survey provided detailed data for:

a) further evaluation of select tools; and
b) hypotheses testing.

Table 3.4 provides a list of the tools that were used and evaluated during this phase of AETE. Results and recommendations pertaining to individual tools are discussed in Chapters 4-6. A major contribution of this portion of the program was hypotheses testing. The contribution of a tool for hypotheses testing was also one of the primary criteria used for tool evaluation and selection. The approach for hypotheses testing is described in more detail below.

Table 3.4 Tools Evaluated in the 1997 AETE Field Studies

Toolbox	Tool	Method
Water Chemistry	Total metals (ICP-MS) Dissolved metals (ICP-MS)	U.S. EPA Method No. 200.8 0.45 μm filtered
Sediment Chemistry	Total metals (ICP-MS) Partial metals (ICP-MS oxide-bound fraction) Simultaneously extracted metals (metal monosulphide fractions)	nitric acid/hydrogen peroxide extraction hydroxylamine hydrochloride cold hydrochloric acid digestion
Effluent Sublethal Toxicity	*Ceriodaphnia dubia* *Pimephales promelas* (fathead minnow) *Selenastrum capricornutum* (algae) *Lemna minor* (duckweed)	Environment Canada 1992a Environment Canada 1992b Environment Canada 1992c Environment Canada, 1999
Fish Tissues	Metal levels Metallathionein	gill filaments whole kidney whole liver dorsal boneless muscle fillet entire gut from small fish
Sediment Toxicity	*Chironomus riparius* *Hyalella azteca* *Tubifex tubifex*	Environment Canada 1997a Environment Canada 1997b ASTM E1384-94A, 1995
Fish Survey	Growth Organ size	total body weight (g) fork length (mm) total gonad weight (g) total liver weight (g) total number of eggs per female
	Abundance Community	total number of fish per standardized effort electrofishing or gill netting (CPUE) total weight of fish per standardized effort (BPUE)
Benthic Invertebrates	Community indicators Fitness parameters	number of taxa at lowest practical level of taxonomy invertebrate abundance expressed per m^2 number of genera in mayfly, caddisfly and stonefly orders abundance of indicator taxa at generic level chironomid mouth deformities

3.4.2 Hypothesis Testing Methods

The detailed methods and results of the hypotheses testing are described in BEAK and Golder Associates (1998c). The methods are outlined below while the results are described under various monitoring tools and in Chapter 7.

The general reasoning behind all of the hypotheses is that a mine "effect" is a measurable difference between reference and exposure locations, and/or a trend is apparent between locations that are exposed to different concentrations of effluent. The hypotheses address either the ability of a particular monitoring tool to detect such an effect (e.g. H5-H8) or the relative ability of two different monitoring tools to detect such an effect (e.g. H1-H4). Hypotheses H9 through H12 address the relative ability of two monitoring tools to detect a correlation between specific predictor (exposure) and response variables (effect), while

Hypothesis H13 addresses the ability of a particular toxicity testing tool to show such a correlation.

The different types of hypotheses required different methods of statistical analysis which are detailed in each of the site reports. It should be recognized that a significant correlation between exposure and response variables does not prove cause and effect. The following subsections summarize the statistical approaches applied for each category.

H1 through H4 - Comparison of Tools for Ability to Detect an Effect

Hypotheses H1 through H4 are tool comparison tests. Tools (response measures) were tested pairwise to determine their relative ability to detect a mine related impact. From a group of comparable tools (e.g. toxicity tests), this comparison allows the selection of the tool or tools that can best measure the impact of mine-related exposure. H1 compares sediment toxicity endpoints between common test organisms, whereas H2 through H4 examine metals and metallothionein in various fish tissues. Specifically for H2 and H3 tissues are the tools for comparison. In H4, the tool comparison is between metal and metallothionein, rather than between two tissues.

To determine whether two monitoring tools differed in their ability to detect mine effects, simple analysis of variance (ANOVAs) were used to identify whether there was a significant area x tool interaction (i.e., two tools showing different patterns of response with exposure level). If there was, then a plot of the interaction was examined to confirm that the pattern was consistent with one tool being a better indicator of mine effects.

For mine sites where gradient designs could be applied, the ANOVAs were used to compare tool effectiveness in two ways:

- by determining if the tools differed with respect to their reference-exposure difference (a larger reference-exposure difference indicates greater effectiveness); and
- by determining if the tools showed a similar linear trend or gradient in response within the exposure area (a stronger trend indicates greater effectiveness).

H5 through H8 - Fish CPUE, Growth, Organ Size and Benthic Community Responses

Hypothesis H5 compares fish catch-per-unit-effort in reference and exposure areas. Hypothesis H6 compares a number of indices collected from benthic and fish communities (e.g., number of taxa, number of individuals, abundance of particular indicator taxa) in the areas compared. Hypothesis H7 examines area differences in weight and length (age adjusted if necessary), and H8 tests for area differences in liver and gonad weight for each sex and fecundity (body weight adjusted if necessary).

Hypotheses H5 through H8 address the ability of a particular community or population index tool (response measure) to show a relationship to mine exposure. For CI designs, a response variable such as fish growth or number of benthic taxa was compared by ANOVA for stations across the two or three areas (e.g. reference, near-field, and far-field) to determine if area means were significantly different and whether the pattern is consistent with a mine effect.

In cases using gradient designs, ANOVAs were used to partition overall variance in the response measure into a number of terms, representing effects of particular interest. The results of testing hypotheses H1-H8 are presented in Chapters 4-6 and are used extensively as a criterion for tool recommendation (e.g. H4: metals vs. metallothionein in fish tissue).

H9 through H12 - Tool Integration hypotheses

H9, H10 and H11 address the **relative** ability of two monitoring tools to detect a mine effect in the form of a correlation between exposure and measured responses. For example in H9, dissolved metal concentrations in water were compared with total metal concentrations to determine whether these two monitoring tools differed in their correlation with a measured biological response such as number of benthic invertebrate taxa or fish abundance.

H10 was tested in a similar manner by correlation of sediment chemistry versus benthic or fish index values.

Hypothesis 11 examines the remaining component of the "sediment quality triad' which is the correlation between benthic indices and sediment toxicity. The toxicity tests include the amphipod (*Hyalella azteca)*, chironomid *(Chironomus riparius)* and oligochaete (*Tubifex tubifex*) on sediment samples from each stream station.

Hypothesis 12 examines the correlation between water and sediment chemistry and metals or metallothionein concentrations in fish.

H13 - Linkage of Effluent Sublethal Toxicity with Benthic and Fish Results

Hypothesis 13 examines the ability of a particular effluent toxicity testing tool to predict a biological effect that has been observed in the receiving environment. This was only quantitatively possible at Heath Steele. At Myra Falls and Dome Mine, this hypothesis was qualitatively evaluated because there was only one level of exposure at Myra Falls, and Dome was not discharging effluent at the time of the survey.

Methods for testing H9-H13 are described in more detail in Chapter 7.

Not all hypotheses could be tested at each site, although the Dome and Mattabi sites were almost complete with 12 hypotheses tested at each site. Table 3.5 summarizes which hypotheses were tested at each of the sites.

Table 3.5 Summary of Hypotheses Tested at Each Mine Site

Hypothesis	Heath Steele	Dome Mine	Mattabi Mine	Myra Falls	No. of Sites Tested
H1: Comparison of Sediment Toxicity Tests		X	X	X	3
H2: Comparison of Metals in Fish Tissues		X	X		2
H3: Comparison of Metallothionein in Fish Tissues		X	X		2
H4: Comparison of Metal vs. Metallothionein in Fish Tissues	X	X	X		3
H5: Effects on the Fish Community - CPUE, BPUE	X		X		2
H6: Effects on Fish or Benthic Communities	X	X	X	X	4
H7: Effects on Fish Growth	X	X	X		3
H8: Effects on Fish Organ Size or Reproduction		X	X		2
H9: Relationship between Water Quality and Biological Components	X	X	X	X	4
H10: Relationship between Sediment Chemistry and Biological Responses	X	X	X	X	4
H11: Relationship between Sediment Toxicity and Benthic Invertebrates		X	X	X	3
H12: Relationship between Metals or Metallothionein in Fish and Metal Concentration in Water or Sediment	X	X	X		3
H13: Chronic Toxicity - Linkage with Fish and Benthos Monitoring Results	X	X*		X*	3
No. of Hypotheses Tested	8	12	12	6	

*qualitatively tested

4. ARE CONTAMINANTS ENTERING THE SYSTEM?

This chapter reviews the tools used to address the first guidance question posed by the AETE program: *Are contaminants entering the system?* It is the most fundamental of questions when undertaking an impact assessment. This question relates to the presence of elevated concentrations of chemical substances in effluent, water and sediments.

The tools discussed in this section are broadly grouped into four categories: a) effluent chemistry, b) effluent acute toxicity, c) receiving water quality, and d) sediment quality. Effluent acute toxicity testing is included in this section because it is an end-of-pipe measurement that provides an indication of effluent quality. Toxicity responses are sometimes evident in samples where none might be predicted on the basis of chemistry alone, and the opposite may also be true. Effluent sublethal toxicity testing tools are discussed in Chapter 6 (*Is there a measurable biological response?*). For the purposes of AETE, sublethal toxicity tests were used, in part, to predict biological responses in the receiving environment.

4.1 Effluent Chemistry

The logical place to begin addressing the first guidance question "*Are contaminants entering the system?*", is liquid effluent discharge. Measuring effluent quality, or chemistry, is currently a federal requirement under the MMLER for a limited number of parameters. Provincial or territorial agencies may add to the list of chemical parameters on a site specific basis for regional Certificates of Approval (C. of A.). Most mines are also required to measure effluent volume on a weekly or monthly basis. This information provides some indication of loading to local watersheds. Effluent quality and quantity are measured at an agreed-upon location, usually at the end of the treatment facility ("end-of-pipe") prior to discharge to the environment.

During the AETE field program, effluent chemistry was measured as part of all three field surveys. In addition, effluent chemistry was measured and reported during the laboratory toxicity screening tests (see Section 4.2). Measuring effluent chemistry was fundamental to the AETE program since it:

- provides an indication of chemical loading to the environment,
- helps scope parameters to monitor in the receiving environment,
- permits analysis of relationship between effluent chemistry and toxicity, and
- permits analysis of relationship between effluent chemistry and in-stream biological responses.

The latter two considerations are discussed in detail in Section 7.0. No literature evaluation of measuring effluent chemistry was undertaken under the AETE program. The basic methods used and parameters measured are described in Toolbox Summary #1.1 (Appendix B).

Modern analytical techniques are such that "scans" of samples can provide results on 20-40 parameters depending on the methodology. The basic parameters to measure will include metals with particular focus on certain elements depending on the mine type and nature of the ore being processed. Some sites may have elevated natural concentrations of arsenic or molybdenum, for example, and these elements should be closely monitored.

Analysis for different substances will often require different sample preservation methods, different sample volumes and different analytical techniques requiring several samples for complete effluent description. During the AETE surveys, the basic effluent parameters were grouped as follows:

- Metals: total and dissolved;
- General chemistry (e.g. alkalinity, chloride, sulphate, hardness, conductivity, full cation and anion analysis and balance);
- Total suspended solids;
- Nutrients: nitrate, nitrite, phosphorous, ammonia, total kjedahl nitrogen (TKN); and
- Total and free cyanide, where appropriate.

Analytical detection limits are generally not a problem with effluents since concentrations of the chemicals of concern are often elevated at the source. Therefore, analytical methods can be used (e.g. standard ICP) that may not be suitable for determining metal concentrations in the receiving environment where lower detection limits are necessary. Whatever method is used, it must be able to achieve detection limits that, at a minimum, are below the effluent discharge C. of A. limits. More discussion on recommended analytical methods, detection limits and quality assurance/quality control (QA/QC) is provided below in Section 4.4.

Effluent samples for chemistry and toxicity testing are generally collected as "grab" samples at the point of collection. However, some C. of A. or provincial requirements may specify that a "composite" sample be collected over a specified period of time, i.e. 24 hrs. Effluent quality can vary temporally for a variety of reasons, and the purpose of a composite sample is to provide a measure of "average" conditions over the sampling period.

The quality and quantity of liquid effluent discharge from a mining operation can vary seasonally depending on operations and climate. In Canada, many mine operations discharge very little, if any, liquid effluent from their tailings basin and holding facility during the winter months. In the case of gold mines for example, natural degradation of cyanide products is very slow during the winter, and it may be difficult to achieve discharge limits. Therefore, the wastewater is slowly discharged during the spring and summer. Other operations will have relatively constant discharge flows. Effluent must also be discharged during the spring to release large quantities of snow meltwater that has accumulated during the winter.

The relative concentration of effluent in the receiving environment will depend upon the effluent volume and nature of the receiving water. Conditions of both these systems can and do vary seasonally. Where effluent is discharged to a lake, the relative concentration and mixing area will depend more on the effluent quantity since lake volume does not naturally change substantially. Where effluent is discharged to a river or stream, the mixing area can change significantly due to seasonal fluctuations in flow.

During the AETE program, exposure to effluent was determined by measuring relative concentration of known effluent parameters (i.e. metals, sulphates, conductivity) in the receiving environment. However, sampling was only undertaken on one occasion and the spatial extent of the effluent mixing zone under different flow conditions was not assessed. Mixing zones can be estimated using dilution modeling and by undertaking in-stream dye tracer studies. For either approach, good hydrological information on seasonal flow patterns is necessary.

In summary, effluent chemistry and quantity are recommended as tools in a routine monitoring program.

4.2 Effluent Acute Toxicity Testing

The first major study of the AETE toxicity testing component was to evaluate potential alternative tools to the commonly used reference test organisms, rainbow trout and *Daphnia magna*. Although both these organisms are generally considered ecologically relevant, there is concern about obtaining timely results. The time for a sample to reach a contract laboratory, complete the test and prepare the results generally involves several days. Recently, a number of commercially available "micro" screening toxicity test techniques have been developed. If alternative micro tests are found to be acceptable they could possibly be used at the mine site to provide routine toxicity data and better turn around time for test results.

Secondly, there are concerns about the cost of the rainbow trout and *Daphnia magna* tests. Therefore, one of the objectives of the AETE program was to evaluate the ability of the alternative micro toxicity tests to provide similar responses to the standard rainbow trout and *Daphnia* tests but at less cost and greater speed.

It should be noted that the term "acute" applies to tests of short duration relative to the life cycle of the organism, e.g., a rainbow trout 96hr test. In these tests, mortality (or survival) is the most commonly measured endpoint. However, a number of the alternative micro tests that were evaluated use acute (short) exposure periods but mortality is not necessarily the endpoint measured.

A total of five alternative short duration tests were evaluated under the AETE program. These included: *Daphnia* IQ test, Microtox acute, Rototoxkit F, Thamnotoxkit F and Toxichromotest. During the AETE program, approximately 65 effluent samples were submitted by 21 participating mines to commercial toxicity testing laboratories and subjected to the alternative tests being considered. The toxicity test results are reported in AETE reports #1.1.1 and #1.1.2 (BAR Environmental 1995). A detailed comparison of the alternative methods and results is provided in AETE Report #1.1.4 (Pollutech Enviroquatics Ltd. 1996).

Each of the alternative tests is very briefly described below. More details on each of the acute toxicity tests are provided in Toolbox Summaries #4.1 to 4.7.

***Daphnia* IQ test:** This test is commercially available as a kit and a standardized protocol is available. The endpoint is based on measuring the uptake of a fluorescent substrate by starved daphnids and subsequent enzyme activity. The time to actually conduct the test is under 2 hours (Toolbox Summary #4.1).

Microtox acute: This is probably the most widely recognized micro test. It is available as a kit and uses a luminescent marine bacteria, *Vibrio fischeri*. The relative inhibition of light production is used as the toxic response. Test duration for this study is 15 minutes. (Toolbox Summary #4.2).

Rototoxkit F: The Rototox F kit comes with cysts of a freshwater rotifer, *Brachiunus culyciflorus*. Cysts are hatched in the lab and neonates are exposed to an effluent dilution series for 24 hours and survival is recorded as the endpoint (Toolbox Summary #4.3).

Thamnotoxkit F: This microtest includes cysts of the fairy shrimp *Thamnocephalus platyururus*, a freshwater crustacean. The cysts are hatched in the lab and within 4 hours the young are exposed to effluent samples for 24 hours. Survival is the measured endpoint (Toolbox Summary #4.4).

Toxichromotest: This test uses a colour endpoint to estimate the concentration of a sample that causes 20% toxicity to a strain of *E. coli* bacteria. The required exposure period is 90 minutes (Toolbox Summary #4.5).

The rainbow trout and *Daphnia magna* tests are described in Toolbox Summaries #4.6 and 4.7, respectively (Appendix B).

The results of each test were evaluated in terms of comparability of sensitivity with rainbow trout, correlation to effluent chemistry, cost, speed and reproducibility. The rainbow trout test was used as the principal benchmark acute lethality test as it has historically been the test most commonly required under various Canadian regulatory programs. Results of the micro test results were statistically compared with:

a) prediction of toxicity results based on effluent chemistry, and

b) the rainbow trout LC50 for the same effluents.

The other criteria were scored and the results ranked for the individual test over the range of effluents sampled (Pollutech Enviroquatics Ltd. 1996).

The evaluation of the alternative tests included extensive data analysis and comparisons of the results. It quickly became apparent that test results, notably sensitivity, differed markedly depending on the type of mine where the effluent was obtained. Therefore, results of each criteria evaluation were presented by mine type. The different mine types included (# of that type of mine participating in study): gold (5); bitumen (1); tin (1); uranium (2); zinc (1); copper/zinc (3); lead/zinc (3); and nickel/copper (5). This division by mine type reduced the power of statistical analysis but was considered to be an important recognition of site specific conditions.

For correlation analysis with effluent chemistry, approximately 27 chemical variables were considered (pH, total suspended solids (TSS), ammonia, conductivity, + metals scan). The rainbow trout results correlated with the concentration of 9 chemical parameters. It should be noted that correlation does not imply causality, especially when multiple elements are present in the effluent. The *Daphnia magna* IQ test and Thamnotoxkit results were correlated with 11 and 10 parameters, respectively, while the other tests, including the *Daphnia magna* acute test, were correlated with less than 8 chemical variables. AETE Report #1.1.4 (Pollutech Enviroquatics Ltd. 1996) also provides an interesting summary of the concentration of each variable for each test where a positive toxic response was measured.

For all types of mine effluent, the *Daphnia magna* IQ test was more sensitive than the rainbow trout test (Table 4.1). Where valid comparisons were possible, the rainbow trout was always more sensitive than either Microtox or the Toxichromotest tests. It is difficult to generalize about other trends in sensitivity due to the rather small sample size for some mine types.

Table 4.1 Summary Comparing Microtests to Rainbow Trout Acute Toxicity Results by Mine Type

Mine type	*Daphnia magna* Acute	*Daphnia magna* IQ	Microtox	Rototox	Thamnotoxkit	Toxichromotest
Gold		*Daphnia magna* IQ More sensitive		Rototox More sensitive	Thamnotoxkit More sensitive	Rainbow trout More sensitive
Bitumen	Rainbow trout More sensitive	*Daphnia magna* IQ More sensitive	Rainbow trout More sensitive	Rainbow trout More sensitive	Rainbow trout More sensitive	
Copper/ Zinc		*Daphnia magna* IQ More sensitive	Rainbow trout More sensitive		Thamnotoxkit More sensitive	
Nickel/ Copper	Rainbow trout More sensitive	*Daphnia magna* IQ More sensitive				Rainbow trout More sensitive
Lead/ Zinc	*Daphnia magna* More sensitive	*Daphnia magna* IQ More sensitive			Thamnotoxkit More sensitive	Rainbow trout More sensitive
Tin		*Daphnia magna* IQ More sensitive	Rainbow trout More sensitive			
Uranium	*Daphnia magna* More sensitive	*Daphnia magna* IQ More sensitive		Rototox More sensitive		
Zinc		*Daphnia magna* IQ More sensitive		Rototox More sensitive	Thamnotoxkit More sensitive	

The final evaluation produced a detailed breakdown of each of the evaluation criteria by each mine type and the interested reader is guided to the original report (AETE #1.1.4) for further information. It was concluded that no one test compared directly with the rainbow trout toxicity test in terms of sensitivity and correlation to chemical endpoints.

The Microtox acute, Rototoxkit and Toxichromotest tests did not satisfy a number of the criteria for comparison with the trout test or reproducibility. The popular Microtox test was determined to be relatively insensitive to metals or mine effluent, therefore, of little value to the industry.

In summary, the Microtox acute, Rototoxkit and Toxichromotest tests are not recommended by AETE for further consideration to assess mining impacts as routine monitoring tools.

The "best" micro test method varied depending on mine type and varied between *Daphnia magna* acute test, the *Daphnia magna* IQ test and the Thamnotoxkit. These three tests were sensitive tools for evaluating the environmental impacts of mining effluents. However, standardized QA/QC protocols must be developed for the latter two tests prior to being included in routine monitoring programs.

Therefore, only the *Daphnia magna* and rainbow trout tests are recommended as effluent acute toxicity tests for routine monitoring.

4.3 Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE)

4.3.1 Introduction

The preceding chapters discussed methods to determine whether a liquid effluent is acutely toxic or not. If the effluent is toxic, the discharger may undertake further studies to determine and eliminate the cause(s) of toxicity. Protocols to investigate the probable causes of toxicity were recently developed by the USEPA (USEPA 1989) and are known as Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) studies. The use of TI/REs is relatively new in Canada and it was considered appropriate to evaluate their application to mining under the AETE program.

A technical review of the TI/REs as applied to the mining industry in Canada was undertaken by ESG International and is reported in AETE report #1.2.5. The objectives of the technical evaluation were to a) complete an overall critical evaluation of the quality of TI/RE data, its benefits and limitations and b) evaluate the utility of the TI/RE strategies in determining and/or addressing aquatic impacts from mining operations.

4.3.2 The TI/RE Process

The general objectives of the TI/RE process are to a) evaluate the potential sources of toxicity, b) characterize the toxicity observed in the sample, c) provide a preliminary identification of the possible sources of this toxicity by evaluating changes that occurred in the toxicity following a variety of chemical and physical manipulations and treatments, and d) ultimately provide measures for reduction and elimination of the toxicants (US EPA 1989). A successful TI/RE will involve the coordination of a multidisciplinary team including toxicologists, chemists, engineers and very importantly, mine personnel.

The TIE portion of the TRE program is divided into three phases:

- Phase I - characterization of toxicity through a variety of effluent treatments (USEPA 1991a);
- Phase II - identification of the suspected toxicants (USEPA 1993a); and
- Phase III - confirmation of suspected toxicants (USEPA 1993b).

Phase I TIEs are considered the next phase of assessment when effluents are identified as toxic in the USA. The Phase I TIE methods were originally developed for use with acute lethality tests using fathead minnows or *Ceriodaphnia dubia* but have been adapted for sublethal and sediment testing (USEPA 1991b,c). In Canada, rainbow trout and *Daphnia magna* are commonly used during TIE tests.

The standard USEPA Phase I effluent characterization treatments involve: filtration at different pH, aeration at different pH, C18 solid phase extraction at different pH, treatment with ethylenediaminetetraacetate (EDTA) and sodium thiosulfate. A significant portion of toxicity observed in industrial effluents is often attributed to pH effects.

Therefore, pH adjustment is used throughout Phase I to provide more information on the nature of the toxicants. Other treatments include passing samples of effluent through columns with carbon or zeolite. In all cases, the toxicity of treated samples is compared to non-treated samples to determine which approach, if any, reduced toxicity.

The TI/RE evaluation for AETE included three stages: 1) literature review, 2) survey of mines across Canada, and 3) review of 5 case studies. The results of each stage are summarized below:

4.3.3 Literature Review

The published primary literature on the TI/RE process was not extensive and even less so for studies directly applicable to mining. However, after speaking to government and private consultants involved in TI/RE studies, this situation was felt to be a reflection of work being conducted for private industry and the results not being widely reported in the published literature rather than a case of TI/REs not being conducted. Exchange of TI/RE information is greatly reduced due to the small amount of published literature. Future sharing of unpublished information is a key element to improve the TI/RE methodology.

4.3.4 Survey

As part of this evaluation, a survey was sent to 119 mine operations across Canada. The survey inquired about a) the results of any TI/RE studies, and b) frequency and cost of such studies. A total of 53 responses were received of which 42 were considered applicable to this TI/RE review. The results are useful in terms of the application of the TI/RE process as well as providing some insight into the nature of toxicity problems within the industry.

The 42 valid survey responses can be categorized as follows: 17 (41%) mines reported their effluent as being non toxic, 24 (57%) mines reported effluents as acutely lethal and 1 (2.4%) reported sublethal toxicity. Of the 25 mines that reported toxic effluents, 9 (36%) reported that toxicity was consistent, while 16 (64%) experienced transient toxicity. Seven mines indicated that a TRE study had been conducted while 17 reported that at least one TIE study had been completed, with another 2 in progress. The majority (16) of studies were Phase I investigations.

Of the 17 completed TIEs, only 6 were considered to be successful in terms of identifying the substance(s) responsible for effluent toxicity. Ammonia was most commonly identified as the cause of toxicity, but toxicity was also related to pH. Interestingly, American laboratories reported that ammonia was not a common problem with mining effluents. Rather, toxicants associated with mines were more often metals, total dissolved solids (TDS) or chemicals associated with effluent treatment (e.g. flocculents).

Several mines reported that TIEs were started on effluents that turned out to be non-lethal, or toxicity did not persist. However, this in itself is valuable information as it suggests the toxicant was not stable and may volatilize or precipitate during storage. The USEPA (1989, 1991a) guidance document clearly indicates that "*TIEs require that toxicity be present frequently enough and endure storage so that repeated testing can characterize and subsequently identify and confirm the toxicants in Phase II and III.*

Therefore, enough toxicity testing should be done to assure consistent presence of toxicity before TIEs are initiated".

In situations where toxicity is sporadic, it may be necessary for mine and laboratory personnel to investigate the use of on-site indicators to predict when the effluent may be toxic. For example, toxicity may be associated with certain effluent parameters (e.g. pH, conductivity) or Total Dissolved Solids (TDS) or a particular operational process that can help focus sampling and scheduling of testing. Once the suspected cause(s) of toxicity is identified, repeat testing on different effluent samples must be conducted in order to account for effluent variability and confirm that the cause of toxicity is the same under all conditions.

Among the companies surveyed, the length of time required to complete a TIE varied from 2 to 12 months and depended upon the complexity of the problem and completeness of the study. A number of respondents indicated the cost was less than $10,000 but a full Phase I TIE was never completed. The majority of TI/RE costs were less than $50,000 but two mines reported spending between $50K and $100K.

4.3.5 Case Studies

The AETE report (# 1.2.5) described in detail case studies (CS) of TI/RE results at five mines in Canada. In CS #1, a copper/zinc mine, ammonia and copper were identified as the primary toxicants. Other possible toxicants (Ag, Al and TDS) were also suspected. The TIE process identified a strategy for reduction of ammonia toxicity to rainbow trout, the main concern of the client. The mine closed, but effluent continues to be discharged and is occasionally toxic.

In CS #2, a uranium mine, the primary toxicant was identified as an aliphatic alcohol (e.g., tridecanol, 1,2 dodecandiol). Modifications were made to the process (e.g., product substitution) and effluent toxicity was eliminated.

In CS #3, a copper/nickel mine, the primary toxicant was identified as ammonia. Secondary toxicants (metals) were suspected, but not identified. Toxicity was reduced by pH adjustment.

In CS #4, a gold mine, copper was identified as the primary candidate for the cause of toxicity. Other possible toxicants (silver and ammonia) were also suspected, but not conclusively identified. Following installation of a treatment plant which included a cyanide destruction process, the effluent remained transiently toxic to both trout and daphnids. Ammonia, produced during the destruction of cyanide, is the suspected cause of trout mortality. Metals may be the cause of daphnid toxicity.

In CS #5, a cobalt/nickel and precious metals refinery, several possible causes of toxicity were suspected, but not conclusively identified. It was hypothesized that sodium levels were sufficient to account for at least 50% of the *Daphnia magna* mortality. Copper, potassium and carbonates were identified as potentially important factors in explaining daphnid mortality. Atypical ion balance was also a suspected cause of daphnid toxicity. Based on the limited available data, it was suspected that periodic peaks in sodium and/or copper concentrations contributed to the sporadic trout toxicity. The standard approach to toxicant identification was not possible since the USEPA Phase I TIE treatments were ineffective at reducing or eliminating effluent toxicity. Subsequent toxicant identification efforts are in progress, but have required the development of innovative methodologies and techniques.

4.3.6 Summary of TI/RE Review

One of the most important benefits of the TI/RE process is that it incorporates the responses of organisms into the assessment of complex effluent mixtures to determine the identity of the substance(s) responsible for toxicity. Attempts to use chemical screening alone to identify substances responsible for effluent toxicity are typically unsuccessful.

The application and benefits from the TI/RE process cannot be realized unless a detailed toxicity study is undertaken. Most mines reported that primary toxicants were easily identified, but secondary causes of toxicity seemed to be based on speculation. Any dissatisfaction with the TIE process was often related to lack of identification of these secondary toxicants, yet many mines reported not going beyond the Phase I TIE.

A number of mines that were unable to identify the cause of toxicity indicated that toxicity was transient or dissipated over time. Determining the cause of transient or non persistent toxicity can be difficult and may require the testing and analysis of a large number of samples.

It must be emphasized that the TIE approach is not standardized beyond Phase I and subsequent studies to identify the specific toxicants require experienced personnel. There is no Canadian protocol to conduct TI/REs. The TI/RE approach does not prove the cause of toxicity but rather uses a weight of evidence approach. Toxicants are also often identified on the absence of contrary evidence (Mount 1997). Even a complete TI/RE may not conclusively identify the source of toxicity. However, a full TI/RE approach can be useful to characterize, identify and reduce the sources and causes of effluent toxicity in many cases.

In summary, the TI/RE approach was not evaluated by AETE as a method to detect an effect and, therefore, is not considered a routine monitoring tool. However, this approach does have application on a site specific basis for more detailed investigation of the cause of effluent toxicity.

4.4 Receiving Water Quality

The accurate determination of receiving water quality is important to: a) measure potential effects of mine effluent discharge on ambient water chemistry, and b) determine if biological responses can be related to water chemistry. The second relationship is explored in Chapter 7.

Water quality monitoring techniques for measuring total and dissolved metal concentrations and their relationship to biological effects were reviewed by EVS Consultants (1997) (AETE #3.1.2). In addition, a review of collection, filtration and preservation methods of surface waters for detection of metals and metalloids was completed by Hall (1998) (AETE #3.1.3). Water samples for chemical analyses were collected in all three AETE field programs. The general sample collection and analytical methods used by the AETE program are summarized in Toolbox Summary #2.1.

There has been an ongoing debate about the role of dissolved, including colloid-bound, metals versus total metals in predicting biological effects in the receiving environment and the ability to consistently separate these fractions through filtration. This topic is discussed at length in the previously mentioned documents.

The need to achieve method detection limits (MDL) equal to or lower than 1/10 of the corresponding CCME (Canadian Council of Ministers of the Environment) or provincial water quality guidelines was important to the AETE program. Also, the relationship between biological effects and MDLs is important as there are direct cost considerations depending on analytical methods used. The EVS report concluded that non-detect levels should be significantly different than effect concentrations using a site specific risk based approach.

While a broad range of water sampling devices are available and well known (i.e. Van Dorn; Kemmerer, Automatic or Composite), the crucial aspects of water samplers are material compatibility and preparation/preservation of samples. At the detection limits presently available for either total or dissolved metals, the prevention of even low level contamination during sampling is extremely important.

Typical sources of contamination include: sample bottles and caps, preservatives, filters, equipment and poor sampling, handling and storage practices. Proper QA/QC measures include clear documentation of all sample collection, handling and storage methods and proper tracking of samples. The use of field blanks and travel blanks also helps to ensure that potential sample contamination is minimized or identified.

4.4.1 Collection, Filtration and Preservation

A review of cost effective protocols for collection, filtration and preservation of surface waters was completed for the AETE program by the Geological Survey of Canada (Hall 1998; AETE #3.1.3). The GSC conducted five discrete evaluations including test tubes, bottles, filter systems for contamination, filter systems for retention of colloids and a stability storage study. The results are briefly reviewed below.

Test Tubes

For ultralow determination of Ag, At, As, Cd, Ca, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Tl, and Zn in water samples, Fisherbrand polypropylene tubes were soaked in 1% HNO_3 for 24 hours and rinsed in water. Their blue polystyrene caps should be avoided when analyzing for Al and Zn unless they undergo vigorous cleaning. A brief rinse with dilute HCl (of same stock as used for analysis) is also recommended for determination of Hg and Se particularly at levels of 1 ppt or less.

Bottles

Five types of bottles were evaluated including:

EEP (Teflon) Nalge #1600, approximately $28 each

HDPE (High density polyethylene) Nalge #2007, $0.90 each

PETG (Polyethylene teraphthalate colpolyester), Nalge #2019, $2.60 each

PP (Polypropylene) Nalge #2006, $1.00 each

HDPE pre-cleaned round (Superfund Analyzed) to meet or exceed EPA specifications, $2.30 each

The least expensive bottle ($0.90 each) made of HDPE was recommended as having the best characteristics and could be used without rigorous cleaning if batches were checked. Pre-cleaned HDPE bottles were not recommended due to unnecessary expense. Polypropylene bottles needed cleaning if Al is a concern. PETG bottles were expensive and required cleaning. The very expensive Teflon (FEP) was not recommended.

Three bottle cleaning methods were investigated: a) simple rinse, b) modified EPA Method 1638, and c) HNO_3 wash as described by Virginia Department of Environmental Quality. The last method was preferred as it was less expensive, quicker and effective.

Filters (Contamination)

Filters were tested for their expected contribution to contamination levels and their ease of use. Twelve 0.45 µm and two 5 µm filters were evaluated for potential contamination levels. The majority of filters were either Gelman or Millipore, the two leading manufacturers. Three types of systems were used as follows: syringe filters, in-line filters and vacuum filters.

Optimum performance was achieved by the syringe filters. The two highest rated were the Acrodisc syringe filter from Gelman and the Sterivex syringe filter from Millipore. Nylon membranes were not recommended due to slowness. The Millex 5 µm syringe prefilter was recommended for samples high in particulate matter

A number of filters were deemed "acceptable" for environmental monitoring. These included the Millicup bottle top with Durapore membrane (vacuum system); the in-line Gelman Aquaprep with Thermopor membrane; and the Aquaprep 250 with Supor membrane. With sufficient rinsing the Gelman syringe GHP Acrodisc; Millipore Millex HV syringe; and the in-line Gelman groundwater capsule were also acceptable as were the Gelman syringe nylon Acrodisc, the Millipore all glass vacuum, the Gelman groundwater capsule and the Gelman Aquaprep 250 for acidic samples.

Filters (Colloid Retention)

It was noted by both EVS consultants (AETE #3.1.2) and by the GSC (AETE #3.1.3) that simple filtration of water by 0.45 µm filter is inadequate for determination of "dissolved" metals. The presence of colloid bound metals in the dissolved fraction of filtered water appears to be the most significant concern. The unknown toxicity and bioavailability of this fraction has led to debate over the importance of dissolved versus total metal concentrations using physical separation with 0.45 µm filtration.

Both water quality and filter characteristics can produce significant variation in recovery of metals. Both inclusion/exclusion of colloidally-associated trace elements in the filtrate as well as dilution and sorption/desorption from filters were indicated as sources of variation. In summary, the current definition of dissolved metals appears to be inadequate.

The Geological Survey of Canada evaluated retention of colloids for a subset of the filters previously tested for contamination. The Millipore Sterivex capsule system or Durapore based alternatives were recommended if the goal was to measure the fraction of an element present in the 0.45 μm fraction. This system exhibited a high and consistent recovery of all 17 elements measured in the study. Maximum retention of colloidal species were found in Gelman's Supor membrane based systems. Since toxicity of colloids is presently still a question, the significance of these results also remains a question. Sorption of Hg appeared problematic for all filter systems and further assessment was recommended.

Preservation Methods

Preservation methods for 16 analytes were also evaluated. Results indicate acidification with 0.4% HNO_3 should maintain Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Tl and Zn in water samples for at least one month at room temperature. Stability of Ag at concentrations of several hundred ppt was questioned. These results were independent of container material. The best preservation for Hg was identified as 0.5% BrCl while 2% HCl or 0.04% $K_2Cr_20_7$ were considered questionable.

4.4.2 Analytical Methods

Established Analytical Technologies

Inductively Coupled Plasma (ICP) techniques are the most commonly used methods for detection of metals and include mass spectrometry (ICP-MS), atomic emission spectrometry (ICP-AES) and optical emission spectrometry (ICP-OES). Methods for analysis of total versus dissolved metals depends on physical separation of the sample through an 0.45 μm filter prior to fixation with a preservative such as nitric acid.

In general, ICP-MS appears to be ideal for water analysis due to its sensitivity (comparable to graphite furnace), simple spectra and ability to obtain isotopic information of elements. While using these instruments is more expensive, on a single element basis, than Atomic Absorption Spectrometry (AAS), the ability to scan multiple elements makes it cost effective.

Alternative Analytical Technologies

Alternative technologies, designed to measure free metal ion concentrations in natural waters, are less commonly used than ICP or AAS. The technologies reviewed by EVS (AETE #3.1.2) included Anodic Stripping Voltametry (ASV), Ion Selective Electrodes (ISE), Ion Exchange Resins (IER), Ion Chromatography (IC) and Bioassay Data. It was concluded that while measuring the free metal ion concentration is an admirable goal, given the difficulties in separation to 0.45 μm fraction consistently and the questionable value of this practice in evaluating toxicity, the use of ICP/AAS techniques should continue until some of the alternatives are further developed or new ones are proven.

4.4.3 AETE Field Survey Results

Documenting water chemistry in the receiving environment was an important component of all three AETE field studies. Detecting a statistically measurable difference in water quality downstream of the mine operation relative to an upstream or reference site was considered a significant effect under the AETE program.

The results of the field programs demonstrated that concentrations of various chemical parameters were consistently elevated downstream of a mine effluent relative to reference areas. Even at sites with reduced discharge, water quality conditions were measurably different below the mine site. The parameters showing differences between areas were often site specific depending on the type of mine, operational processes and treatment facilities as well as surrounding conditions.

Conventional water chemistry parameters that were generally elevated below the mine discharge included conductivity, chloride, sulphate, Total Dissolved Solids (TDS), Total Kjeldhal Nitrogen (TKN), ammonia, nitrate, calcium, hardness and bicarbonate. The concentrations of some cations related to hardness can often be attributed to materials used for effluent treatment. Cyanide (total and free) was sometimes present in effluent and elevated in downstream waters. Conductivity is easy to measure in the field and may be used as an indicator of general effluent mixing and dispersion in the receiving environment.

The 1995 Pilot Study observed that the ICP-MS provided for analysis of more elements than ICP, and typically provided for detection limits which are 1,000 fold lower than provided by ICP. Thus, ICP-MS solved detection limit problems associated with ICP (BEAK 1996).

During the 1996 surveys, there were some situations where the concentration of dissolved metals was higher than the total metal concentration. From a mass balance perspective, this situation is theoretically not possible and the results were immediately suspect. It was subsequently determined that some metals and other elements were leached from the filters used in the field. Commercially purchased distilled water was also a source of contamination to some of the field blanks. This experience reinforced the value of having a rigorous QA/QC program in place to minimize sample contamination, and to detect possible contamination or laboratory errors when it does occur.

At the Dome and Val d'Or AETE sites, the concentration of a metal or parameter was sometimes higher in the reference area relative to the downstream exposure area. This situation could usually be attributed to contamination from old tailings deposits or other sources of contamination. Similarly, the concentration of a particular metal at some reference areas approached or even exceeded the relevant *Canadian Water Quality Guideline* for that substance. That situation may also be due to historical upstream contamination or the presence of highly mineralized water in the region due to natural weathering of the parent bedrock. Both situations emphasize the need for careful choice of reference areas and a good understanding of other potential confounding factors in the watershed.

At all mine sites during the 1996 and 1997 surveys, water chemistry sampling revealed that metals and other contaminants (e.g. nutrients, ammonia) were "getting into the system". This was demonstrated by elevated concentrations of these parameters in the exposure area relative to the reference area(s). The AETE program was particularly interested in determining if a) the exposure-reference differences were more distinct using "total" versus "dissolved" metals, and b) the linkage between water chemistry and in-stream biological effects was influenced by the choice of either "total" versus "dissolved" metals (e.g. testing Hypothesis 9).

Detailed results of the 1995, 1996 and 1997 field surveys are provided in the individual site reports and only the significance of the findings are presented in this Synthesis

Report. Similarly, the results of the hypothesis testing are provided in the individual 1997 site reports identified in Section 3.4.1.

Metals that exceeded *Canadian Water Quality Guidelines* (CWQGs) downstream of some of the mine sites included As, Cu, Pb, Se, Sn, Al, Cd, Fe and Zn. The concentrations of many nutrients and other parameters were consistently elevated downstream of a mine site but there are generally no guidelines for these other parameters. Figure 4.1 illustrates the mine-related gradient for Cu at the Dome Mine. In contrast, Arsenic is actually elevated in the upstream reference due to historical tailings deposits.

The 1997 survey results indicated that both total and dissolved metal concentrations were effective at demonstrating that contaminants were entering the system (Table 4.2). At most mines a high proportion of metals was in the dissolved form. Consequently, there was little difference in identifying mine-related trends between either total or dissolved metals, and either would be equally effective as a monitoring tool based on this criteria alone.

Table 4.2. Total Versus Dissolved Metals As Indicators Of Exposure *

Mine Site	Total Metal	Dissolved Metal	Comments
Dome Mine	√	√	There were increased concentrations of total and dissolved Cu, Mg, Se, Ag, Co, Ni and K at all river exposure stations. All metals detected above MDL were elevated in exposure lake. Total and dissolved metals were equally effective in demonstrating exposure.
Heath Steele	√	√	There was a gradient in total and dissolved Zn, Cd, Cu, Pb, Fe and Al in exposure area. Dissolved and total metal concentrations were similar in effectiveness as indicators of exposure.
Mattabi Mine	√	√	There were increased concentrations of total and dissolved Zn, Cu, Pb and Cd in exposure area. Total and dissolved concentrations approximately equal in reflecting elevated metal concentrations.
Myra Falls	√	√	Increased concentrations of most total and dissolved metals in exposure area. Total and dissolved equally effective in demonstrating exposure.

*	Exposure in this context represents presence in the system and is not related to bioavailability
√	Effect demonstrated
X	Effect not demonstrated
P	Effect partially demonstrated
MDL	Method Detection Limit

For dissolved metals there is some additional sampling time and effort required to filter water samples in the field. In addition, there are additional QA/QC measures for checking the filtering process. However, the actual laboratory cost to analyze a filtered or unfiltered sample is the same. During the 1997 survey, some of the equipment (syringes) required for ultra-trace filtering was difficult to obtain in Canada. The importance of proper filtering equipment was highlighted by experiences in the 1996 field program and the GSC technical evaluation (AETE report # 3.1.3). Therefore, extra equipment, cost and effort is required to obtain a proper filtered water sample.

Another consideration in comparing the value of total versus dissolved metal concentrations is that for the majority of metals, both federal and provincial water quality guidelines are based on total metal concentrations. Therefore, total metal levels must be measured for comparison with the relevant water quality guidelines.

In summary, the measurement of total metal (or other analyte) concentration in receiving waters is recommended for routine monitoring purposes. The measurement of dissolved metal concentrations may also provide useful information in detailed site specific studies.

4.5 Sediment Quality

Sediment quality is a frequent component of many environmental monitoring programs. Chemical substances released from a liquid effluent may settle or precipitate onto the sediments which can affect quality of the habitat. Sediment quality can also be altered through physical changes such as deposition of fine particulate and organic matter.

Measuring sediment quality helps identify which contaminants are entering the receiving system due to a mine discharge. Sediment quality is considered to be a better integrator of average long-term environmental conditions than single-event water chemistry samples. The information on sediment chemistry can also be related to sediment toxicity and biological responses in the receiving environment. In particular, the benthic community is greatly influenced by sediment quality.

Sediment chemistry was measured during each of the three AETE field surveys and the general procedures and methods are described in Toolbox Summaries #3.1 to 3.6. No literature evaluation of sediment chemistry was undertaken as part of AETE.

During the AETE surveys, the basic sediment characteristics measured were grouped as follows:

- Metals: total and partially extractable,
- General chemistry (e.g. potassium, chloride, sulphate),
- Physical characteristics: particle size, total organic content (TOC), and
- Nutrients: nitrate, nitrite, phosphorous, ammonia, TKN.

Analytical detection limits are generally not a problem with sediments since concentration of the chemicals are often elevated relative to water. Therefore, analytical methods can be used (e.g. standard ICP) that may not be suitable for determining metal concentrations in receiving waters.

During the AETE field programs, considerable attention was devoted to locating depositional sediments for the purpose of collection and chemical analysis. It was felt that depositional areas within the receiving environment would more accurately reflect historical loading from mine discharges. Sediment samples were generally collected with an Ekman grab and the top 2-3 centimetres of substrate of each sample removed for analysis. Composite samples were prepared by mixing the surface layers of multiple grab samples. Composite samples were used to account for significant heterogeneity that can be present in sediments.

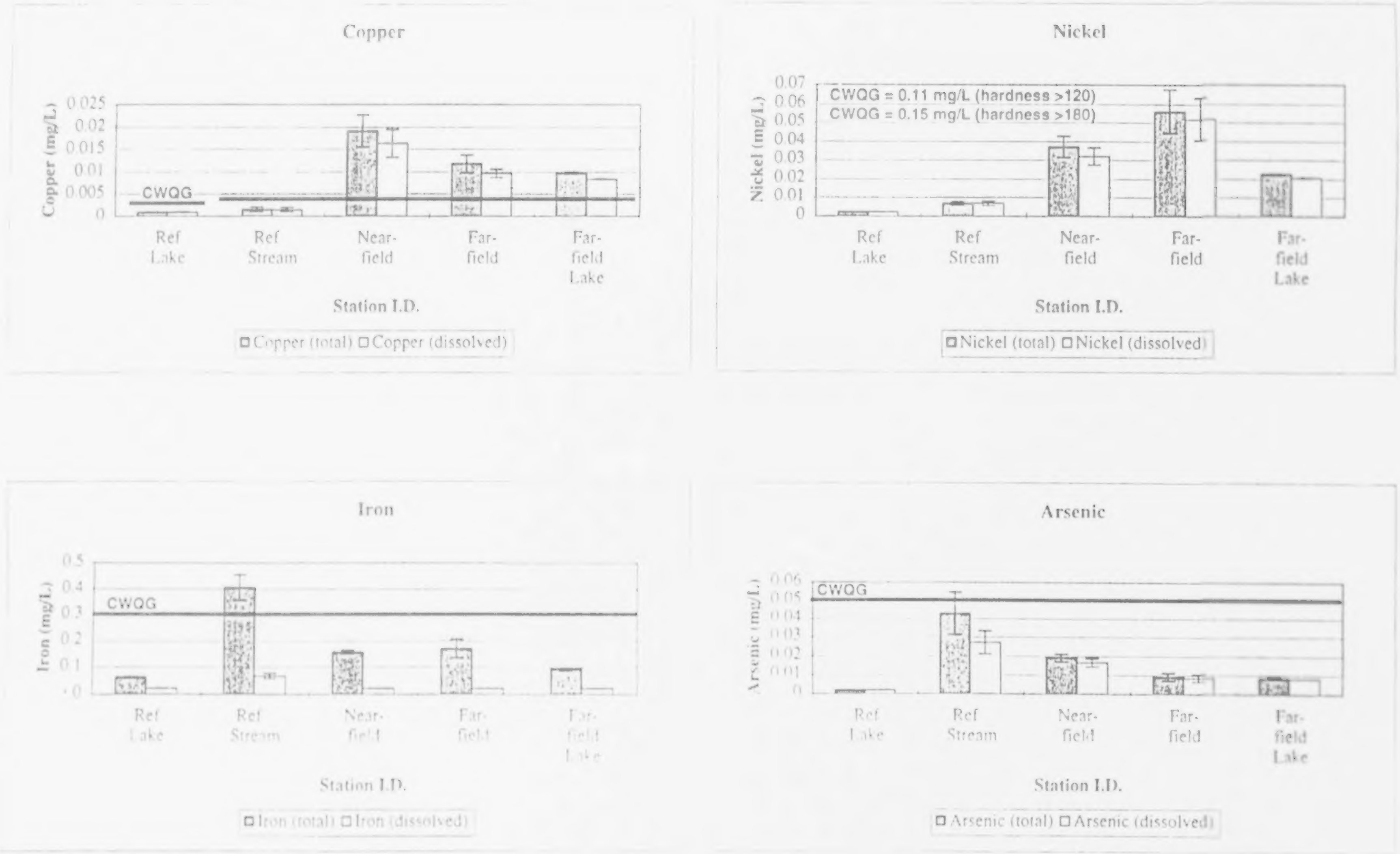


Figure 4.1 Mean Total and Dissolved Metal Concentrations at Reference and Exposure Areas, Dome Mine, October, 1997 Area Means (± 1 S.E.). CWQG = Canadian Water Quality Guideline. Note – CWQG varies for Copper and Nickel in response to water hardness

The concentration of metals in sediments is known to be highly influenced by the proportion of fine particulate matter and organic content of the sample. Smaller particles and organic material have a higher affinity and more binding sites for metals than coarser grained material. Therefore, total metal concentrations tend to be higher in fine organic substrates, with all other factors being equal. To account for this influence, metal levels in sediments can be normalized for particle size or organic content when comparing results between areas. This procedure is outlined in the 1996 field reports. Another option is to sieve sediments through a 63 µm mesh screen to reduce variability. This was not undertaken during AETE but should be considered.

4.5.1 Pilot Study Results

Comparison of Surficial Sediment Mapping Techniques (Toolbox Summary #3.1)

Sub-bottom acoustical profiling methods were compared with more conventional bottom grab sampling combined with the use of a standard sonar unit. The results suggested that sediment characteristics were identified in more detail by sub-bottom profiling than by sonar/grab sampling methods. However, results of the sub-bottom profiling work were far more expensive. Confirmatory cores with up to almost 1 metre penetration also showed that the sub-bottom profiling was prone to misinterpretation of sediment types. In terms of bathymetry mapping, both the sub-bottom profiler and conventional sonar yielded comparable results.

It was concluded that bottom characterization using conventional sonar techniques, supplemented with grab sampling, is generally the more cost-effective approach for identifying sediment depositional areas. It is in these zones where sediment geochemistry and bioassessment studies should be carried out.

For routine monitoring programs, location of depositional sediments and mapping habitat by grab sampler is recommended. This can be accompanied by conventional sonar.

Evaluation of Sediment Coring Methods (Toolbox Summary #3.3)

Sediment cores were only collected during the preliminary 1995 field program to examine which coring devices may be most appropriate. Sediment cores may be useful to measure profiles of metals or other chemicals to provide an indication of pre-mining baseline concentrations. However, they were not considered by AETE to be necessary to detect an effect from a mine effluent. A comprehensive and excellent guidance document on the methods of collecting sediments for chemical and biological testing is provided by Environment Canada (1994).

Three coring devices were compared during the 1995 survey: Hornbrook, Alpine and K-B corers. The study concluded that any of these core samplers could be used in environmental monitoring. However, the gravity type corers allowed for more detailed measurement of sediment profiles. They also had less risk of contamination of the deeper sediments during collection when compared to the Hornbrook sampler.

Sediment cores are not recommended as a tool for routine monitoring. However, sediment cores are useful to provide a depositional history of an area, or to establish background metal concentrations where a reference area is difficult to locate. Therefore, they may be useful in more detailed monitoring programs.

4.5.2 Sediment Pore Water

A technical evaluation of sediment pore (interstitial) water for chemical and biological testing was undertaken by Burton (1998; AETE report #3.2.2a). No field evaluation of sediment pore water was undertaken by the AETE program.

Pore water is defined as the water occupying space between sediment or soil particles. It is often isolated to provide either a matrix for toxicity testing or to provide an indication of the concentration and partitioning of contaminants within the sediment matrix. Metals in pore water may largely represent the biologically available fraction in sediments. Some studies suggest that the primary toxicity of a chemical in sediments is correlated to the pore water concentration (Di Toro *et al.* 1991).

The nature of sediments at the study site can largely influence the usefulness of pore water measurements. Sediments which are either very coarse grained or hard, compacted clays, will not likely have pore waters that are significantly contaminated. Therefore, sampling of pore waters should be restricted to sediments ranging from sandy to non-compacted clays.

Sediment pore water can be isolated using either laboratory or field (*in situ*) approaches. Laboratory methods for collection of pore water from sediment include: a) centrifugation, b) pressurization, or c) suction. Field collection using "peepers" is the most accurate method to obtain representative samples. Peepers are small diffusional chambers with membrane or mesh walls, filled with site water, gels or nonpolar solvents which are buried in the sediments and allowed to equilibriate with the surrounding pore water. The chambers are left in place for 2 to 20 days.

A variety of methods have been used to predict the biological effects of metals from metal contaminated sediments. These include the normalization of sediments for particle size, organic content or extractable fraction of metals using AVS (Acid Volatile Sulphides) and SEM (Simultaneously Extracted Metals). When the SEM fraction exceeds the AVS fraction (e.g. SEM:AVS ratio >1) then the free metal may be present in the pore water at levels adequate to cause acute toxicity. Many studies have shown that acute toxicity of benthic organisms is strongly correlated with pore water chemistry when concentrations exceed their lethal thresholds.

If sediments are anoxic, as most depositional sediments are below 2 cm in depth, then all steps involved in sample collection and processing should be conducted in an inert atmosphere or with limited exposure to prevent oxidation and subsequent sorption/precipitation of reduced metal species if metal speciation is of interest. When anoxic sediments are exposed to air, volatile sulphides may also be lost which may increase the availability (and toxicity) of sulfide-bound metals. Finally, pore water samples undergo rapid chemical changes giving a storage life of only hours to days.

There is a relatively large amount of literature describing toxicity testing with pore water (Burton, 1998). However, it is not as extensive as whole sediments, and there are few standardized methods for toxicity testing of freshwater organisms with pore water.

Environment Canada (1992a) has two standard methods using pore water for Echinoids (Sea Urchins and Sand Dollars) and luminescent bacteria. The AETE Technical Evaluation concluded that pore water toxicity testing in the laboratory is reasonable, if samples are collected and processed properly and the bioassay exposures are realistic. Burton (1998) further recommended that a field demonstration project at geologically diverse mining sites should be conducted to evaluate the utility of pore water toxicity testing.

Based on this analysis, AETE does not recommend the use of pore water for either chemistry or toxicity testing for routine mine monitoring. However, porewater chemistry and toxicity may be useful for more detailed investigation on a site specific basis.

4.5.3 Field Survey Results and Sediment Tool Recommendations

Metal concentrations in sediments were measured in all field studies to determine mine effects. In the Pilot Study (1995), sediment partial extraction results were compared to total extraction results for selected stations. Partial extraction generally extracted most of the cadmium, little of the copper, about half of the arsenic and zinc, and about 30% of the iron and nickel.

Principal Components Analysis (PCA) demonstrated that station groupings based on full and partial extraction chemistry were similar and both were similar to station groupings based on benthic communities.

The study concluded that full extraction of metals coupled with Total Organic Carbon (TOC) and grain size analyses was better able to detect significant differences with greater power among reference, near-field and far-field exposure areas.

During the 1996 surveys only total metal concentrations in sediments were measured. The metal concentrations were normalized for differences in organic content and particle size. The data demonstrated clear increases in the concentration of several key metals (As, Ni, Zn, Cu, Cd) downstream of mine discharge. At some sites a clear sediment gradient was not observed, or it was difficult to match depositional sediments in the exposure and reference areas. This information was used to identify candidate sites for more detailed analysis in 1997.

During the final field surveys in 1997, sediments were analyzed for:

- total metals (nitric acid/hydrogen peroxide extraction method),
- partial metals analysis using a hydroxylamine hydrochloride procedure which is designed to solubilize amorphous Fe and Mn oxyhydrides, along with associated trace metals, and
- Acid Volatile Sulphide (AVS) and Simultaneously Extracted Metals (SEM).

Total and partial metal levels as well as the SEM/AVS ratio were compared for their ability to detect trends in exposure to mine effluent. This comparison is discussed further below.

In addition, these sediment variables were examined for their ability to predict sediment toxicity (H1), or their linkage with biological responses (H10). Sediment chemistry was also used to examine the linkage between exposure to mine effluent discharge and metals or metallothionein (H12) in fish tissue. The relationships between these variables are examined in Chapter 7.

The total concentration of several metals exceeded the Canadian Interim Sediment Quality Assessment Values (CISQAVs) at each of the exposure sites in 1997. The CISQAVs include both a Threshold Effect Level (TEL) and Probable Effect Level (PEL), with the latter being higher than the former. Metals that exceeded the PEL in some instances included As, Cd, Cu, Pb, Hg, Ni, and Zn. At some sites the upstream reference sediments contained levels of Cr, Zn or As that exceeded the PEL.

Table 4.3 summarizes the observations of sediment chemistry tools as indicators of exposure to mine effluent. In general, total metal levels were the most consistent indicator of exposure to mine effluent, and also showed a stronger gradient compared with partial metal levels. The SEM/AVS ratio only showed a mine-related trend at one (Myra Falls) of the three sites where it was tested. Therefore, the SEM/AVS ratio was not considered effective as an indicator of exposure to mine discharge. The laboratory analysis for SEM/AVS ratio is approximately $320 per sample, which is substantially higher than standard sediment chemistry measurements.

Table 4.3. Comparisons of Sediment Chemistry Tools (Total and Partial Metals, and SEM/AVS Ratio) as Indicators of Exposure

	Sediment Chemistry Tool			
Mine Site	**Total Metal**	**Partial Metal**	**SEM/AVS Ratio**	**Comments**
Dome Mine	√*	√	X	Mine-related trends in total Cu, Ni, Co, Fe, Mg, Ag and partial Ni, Cr, Co, Cu, Fe and Mo. Trend stronger in total metals. SEM/AVS ratio did not show a mine-related trend.
Heath Steele	√	NT	NT	Periphyton used as a surrogate for sediments. Cu, Cd, Pb and Zn all showed reference-exposure area differences. Exposure gradient seen for Pb and Fe.
Mattabi Mine	√	P	X	All metals higher in exposure area. Gradients in exposure area evident, particularly for total Zn, Cu, Cd and Pb, but were weaker for partial metals and not evident for some partial metals. No clear difference in SEM/AVS ratios between reference and exposure areas.
Myra Falls	√*	√	√	Most metals higher in exposure area. Gradient in exposure area evident for total Zn, Cu, Cd, Mo, Ag and As, but only for Cd, Cu and Zn for partial metals. SEM/AVS ratios were higher in exposure area than reference, and showed mine-related trend.

NT - Not tested.
√ - Effect demonstrated.
X - Effect not demonstrated.
P - Effect partially demonstrated.
* - More effective at demonstrating an effect.

The laboratory cost of analyzing total or partial metal concentrations in sediments is about $100 each by ICP-MS. However, sediments for partial metal analysis must be frozen and subject to extraction procedures. Therefore, the field crew must have access to either a freezer or dry ice which may be problematic in some locations. In addition, both federal and provincial sediment quality guidelines are based on total metal concentrations.

In summary, AETE recommends that measuring total metal concentrations in surface sediments is a suitable tool for a routine monitoring program as an indicator of contaminants entering the system.

5. MONITORING TOOLS: ARE CONTAMINANTS BIOAVAILABLE?

The bioavailability of metals to aquatic organisms is governed by numerous geochemical and biological factors. The simple presence of a metal in water or sediments does not mean that it is bioavailable. Biological response, particularly to metals, is governed by the bioavailable fraction.

The speciation and geochemistry of metals is complex and entire texts are devoted to the subject. Metal bioavailability is governed by ambient environmental conditions of water and sediment chemistry including pH, redox potential, carbonates, presence of complexing agents (e.g. DOC, clay, TSS) and temperature. Metals in contaminated sediments may affect benthic organisms either a) indirectly by metal partitioning into the ambient water, or b) directly by ingestion of sediments into the gut and assimilation into tissues. Both pathways must be recognized and resolved for a full understanding of exposure pathways (Luoma 1996). Excellent reviews on factors affecting metal availability are available (Bresonik *et al.* 1991; Campbell and Tessier 1996).

The purpose of the tools discussed in this section is to estimate the bioavailability of metals by directly measuring metal concentrations in tissues of the various aquatic organisms that may be exposed to mining effluent. The purpose of measuring total versus dissolved metal levels in water (Section 4.4) was also designed to partially address this issue. However, metal concentrations in water are highly variable and often at or below detection limits for routine measurements. Therefore, establishing a relationship between metal levels in water and/or sediments and organisms is useful as an indicator of ambient metal exposure and bioavailability.

The use of metallothionein (MT) is also included in this group of tools as a measure of metal bioavailability. It could be argued that MT induction is a biological response and more appropriately belongs in Chapter 6. However, a component of the AETE field program was to assess the validity of MT as an indicator of exposure to metals. Therefore, MT is discussed in this section. The linkages between total and dissolved metals in water and MT and tissue metal levels are discussed in Chapter 7 under Hypothesis 12.

The groups of organisms discussed in this review include aquatic plants, molluscs and fish. The AETE program did not evaluate metal uptake and accumulation in benthic invertebrates other than bivalve molluscs.

5.1 Tissue Metal Levels

5.1.1 Aquatic Plants

The use of metal analysis in macrophytes, phytoplankton and periphyton was reviewed for AETE by St-Cyr *et al.* (1997; AETE report #2.3.2). These were ruled out as potential tools for AETE, however, metal uptake in periphyton was tested at Heath Steele during the 1997 field program (BEAK 1998b). The primary reason for examining metal uptake in periphyton in that study was the absence of suitable sediments for metal characterization. The review by St-Cyr *et al.* provides a good introduction and overview of metal uptake by these three groups of aquatic plants. Brief overviews of these methods are provided in Toolbox Summary #5.1.

Submerged macrophytes offer some appeal as potential biomonitors as they are not mobile, have ecological relevance, are widespread and relatively easy to collect. Rooted plants can accumulate metals via their roots as well as direct absorption and adsorption from the surrounding water column. Metal accumulation in plants is affected by at least three factors:

- Plant species,
- Tissue sampled, and
- Season.

Different plant species display substantial variability in metal bioaccumulation based on physiology and growth characteristics of the plants. In fact, the use of some emergent aquatic plants such as cattails and water hyacinths has been promoted for phytoremediation of metal-contaminated water and sediments. In addition, there are often distinct differences in metal concentrations between roots, stems and leaves of macrophytes, with roots generally containing higher levels. Microbial films on root external surfaces can also affect the measured concentrations. In temperate regions, macrophytes undergo seasonal periods of growth and dieback which can substantially affect metal levels, particularly in the above-sediment portions of plants. Therefore, species, tissue sampled and seasonal variability must be accounted for in any potential monitoring program.

Metal uptake in aquatic macrophytes has been measured in numerous environmental research studies related to mining as well as acid deposition effects. However, the use of macrophytes on a regular basis has not been widely endorsed by regulators or industry. Campbell *et al.* (1985) reviewed 105 case studies where metal concentrations were determined in both aquatic plants and the adjacent sediments. In 65% of the studies there was no correlation between these two parameters. A possible simple explanation for this finding is that researchers typically measured **total** metal levels in both the plants and sediments, whereas the plant metal level may only be reflecting the bioavailable portion in water and sediments.

Metal uptake by phytoplanktonic species of algae has been studied both in the laboratory and in the field. Bioconcentration has been reported for numerous species, but a direct relationship between waterborne metal levels and accumulation in the algae is generally lacking. The tremendous species variability associated with community structure in natural populations largely limits this group's potential as a tool for monitoring metal levels.

Periphyton consists of a complex community of micro-algae and bacteria on the surface of rocks or other substrates. This community can be an important primary producer in rivers or littoral zones of lakes, and is a functional interface between the substrate and surrounding waters. Because periphyton are sedentary they have been considered good potential indicators of local water quality conditions (Clements and Kiffney 1994). However, this potential may be more directed toward community structure and biotic indices. Newman *et al.* (1985) concluded that determination of metal accumulation in filamentous algae may be an insurmountable problem for biomonitoring studies.

Samples of periphyton were collected in each of the eight survey reaches at Heath Steele during the 1997 field survey. Samples were scraped from rocks for metal analysis and taxonomic evaluation. Levels of copper, cadmium, lead and zinc all showed differences between reference and exposure areas. An exposure gradient was also observed for lead. However, the concentrations of several key metals including cadmium, copper and zinc often displayed considerable variability between samples within reaches (BEAK 1998).

Further research and studies are needed to clearly demonstrate a relationship between plant tissue levels and ambient environmental loading, either in water or sediments. Furthermore, standardized protocols for collection, species used, sample handling and preparation are required.

The use of metal levels in aquatic plants is not recommended as a routine monitoring tool.

5.1.2 Molluscs

The general use of molluscs as biomonitoring tools was reviewed in AETE Report #2.31. That report is divided into two parts; Part I is a Technical Evaluation (Stewart and Malley 1997) while Part II is considered a Critical Evaluation (Salazar 1997). The former is a broader review of the scientific literature and summary of five case studies using bivalves as biomonitors, while the latter part provides a more pragmatic look at the practical and technical logistics of using molluscs as biomonitors. Metal analysis of mollusc tissues is described in Toolbox Summary #6.1. The analysis of metals in mollusc tissues was not undertaken in any of the AETE field studies.

Molluscs do meet several criteria that make them suitable as biomonitors:

- they are relatively non-mobile so exposure is representative of the study area
- they are abundant, widely distributed and easy to sample and identify
- they are large enough to provide sufficient tissue for analysis
- they are relatively hardy and tolerate a wide range of conditions
- they are shown to accumulate metals

The caveat to the last criterion, of course, is that tissue metal levels must show a correlation with ambient metal loading and concentrations for the organism to be at all useful as a biomonitor. Field studies have shown that concentrations of some metals (Cu, Pb, Zn) in tissues of bivalves are correlated to relatively easily extractable fractions rather than total sediment metal concentrations (Tessier *et al.* 1984).

Bivalves are capable of accumulating metals, however, the actual bioconcentration factor (BCF) is generally not high for several metals of interest to the mining community. Stewart and Malley (1997) summarized reported BCFs for Cd, Zn and Cu from a number of studies reflecting a wide range of species. The general range of BCF ratios from tissue:sediment were as follows: Cd; 0.1-33.0: Zn; 0.5 - 21: and, Cu; 0.1-4.5. In many cases the tissue metal levels were actually less than that in sediments. The authors warn that BCFs can be potentially misleading and should not be used to describe the relationship between tissue metal concentrations and environmental exposure.

Metal uptake in bivalves is influenced by a number of biological variables including species, age, size, growth rate, sex and reproductive status and behavior. Analysis of specific tissues versus whole body will also affect metal levels and results observed. Some studies have related metal concentrations in molluscs to biological responses such as growth rate, but in field situations it is difficult to separate the effects of metal exposure from other environmental influences.

Transplanted and/or caged molluscs have often been used in metal bioaccumulation studies to simulate indigenous populations. Molluscs can be transplanted to exposure sites from either reference areas or obtained from commercial sources. Salazar (1997) outlines many of the practical considerations of working with this group of animals and provides estimates of levels of effort and cost. This group of organisms has apparently been successfully used extensively in the United States for biomonitoring purposes.

Stewart and Malley (1997) cautiously support the use of molluscs as indicators of exposure to metals, but recommend that molluscs should not be used as stand alone tools. While Salazar (1997) more strongly endorses the value of molluscs as indicators, he also recognizes the need to develop standardized monitoring protocols and recommends further research be undertaken to validate the approach.

In summary, molluscs appear to hold potential as biomonitors of metal exposure and bioavailability in mining studies. The use of caged or transplanted organisms may prove useful in situations where it is difficult to obtain adequate numbers of fish or indigenous molluscs for tissue analysis. However, tissue analysis of molluscs was not examined in the AETE field surveys, nor was the cost-effectiveness of the tool evaluated.

AETE does not recommend mollusc tissues as a suitable tool in a routine monitoring program. However, it may be useful for more detailed site specific investigations.

5.1.3 Fish

Metal levels in fish tissue have been measured extensively as part of research and routine ecological monitoring studies around the world. There exists a very extensive body of literature on virtually every aspect of this topic with many excellent reviews available (e.g. Luoma 1983; Newman and McIntosh 1991; Roesijadi and Robinson 1994). In Canada, determination of fish metal levels is often a required component of any baseline study for a new or proposed mine, and subsequent periodic monitoring of fish tissues is often required under the mine's operating C. of A. In the past, the value of the metal levels data has often been limited due to small sample sizes, improper tissues sampled, poor detection limits and inadequate data interpretation. However, these problems can be overcome to provide meaningful and useful monitoring data.

The use of metal analysis of fish tissues as a monitoring tool was reviewed in the AETE program by EVS Environment Consultants (1998; Report #2.2.3). An overview of fish tissue metal analysis as a tool is provided in Toolbox Summary #7.1 In addition, fish tissues were collected and analyzed in each of the three AETE field studies. During the 1997 program, fish tissue concentrations were subsequently compared with metal levels in water and sediment. These relationships are explored and discussed in detail in Chapter 7.

In addition to ambient metal concentration, uptake and accumulation of a metal is governed by the form of the metal and physiology of the organism. Ecological factors including trophic status, diet and feeding strategy can also have a profound influence on metal uptake and retention. Metal accumulation in fish tissues is a result of several processes including metabolism, redistribution and storage to specific tissues and excretion/depuration rates. The relationships between metal concentrations in tissues and the environment can differ substantially among metals, species and tissues.

Some studies have measured metal concentrations in whole fish, but this technique should be limited to smaller specimens (e.g. < 10 cm). If using whole fish or viscera, potential contamination by gut contents must be considered. For larger fish, analysis of specific tissues is usually conducted and is the recommended approach. Dorsal muscle has traditionally been the tissue of choice for metal analysis. Fish muscle offers the advantage of ease of sampling and collection, and it also represents that portion of the fish that is most frequently eaten by humans if consumption of contaminated fish is a concern. However, it is now recognized that mercury is virtually the only element that accumulates in fish muscle tissue. Since mercury is also a known neurotoxin, fish muscle should continue to be collected and analyzed where mercury contamination is known or suspected.

Other fish tissues that have been analyzed for metal uptake include blood, gonads, bone, spleen, brain, gill, liver and kidney. Substantial variability among tissues has been observed. Of these tissues, gill, liver and kidney appear to have the most potential for providing an estimate of the exposure and bioavailability for several metals. Blood and bone tissues may reflect exposure to Pb (Hodson *et al.* 1984) and might be considered if Pb is the primary element of concern. However, these two tissues are not routinely collected and are not considered the site of accumulation for most other metals.

During the 1995 AETE field program, metal levels were determined in various tissues (gills, kidneys, liver, muscle) of larger fish specimens and the whole gut of smaller fish. The primary fish species were northern pike and white suckers. There was substantial data variability, with fish from the Exposure area containing greater metal levels than fish from the Reference area in 9 of 13 comparisons using pike or sucker. The lack of a complete difference in tissue metal levels between Exposure and Reference areas in that study may be attributed to the little or no difference in water metal concentrations between collection areas (BEAK 1996).

The results revealed no significant difference in tissue metal levels between males and females within a species. However, there was a significant positive relationship between the liver concentration of Cd, Ni, Cu, Zn, Pb and Hg and age of white suckers.

During the 1996 field evaluation, metal levels were measured in fish from 5 of the 7 candidate sites. Various species sampled included sculpins, white suckers, pearl dace, Northern Redbelly dace and juvenile Atlantic salmon. The tissues sampled included gill, liver, kidney, and viscera for small fish. Both metal and metallothionein analysis were conducted by the Freshwater Institute in Winnipeg, Manitoba.

At 2 of the 5 sites (Sullivan, Heath Steele), fish tissue metal levels were not different at the Exposure site relative to the Reference area (Table 5.1). The lack of a clear effect at Heath Steele may be partially due to fish migration between the Reference and Exposure areas, whereas the results at Sullivan mine are not easily explained.

At the remaining three sites in 1996 (Dome, Onaping/Levack and Gaspé), tissue metal levels were greater at the Exposure area relative to the Reference area (Table 5.1). At Onaping, metal exposure was reflected in gill and liver (but not kidney) of white suckers. It should be remembered that the purpose of the 1996 surveys was a preliminary reconnaissance for evaluation of candidate sites, not for detailed evaluation of individual tools. Therefore, sample sizes for metal (and MT) analysis were generally small (< 5/area) which limited data analysis and interpretation at most sites.

Exposure to mine discharge was revealed by metal levels in fish tissue at each of the three sites (Dome, Heath Steele and Mattabi) surveyed in 1997 (no fish were collected at Myra Falls). There was variability in the effect using different tissues between sites. For example, gill tissues from white suckers at Mattabi showed a mine-related trend, while gill tissue from perch at the Dome mine was unresponsive. Liver provided some responses at both sites but for only select metals (Se, Mo, Ni). Muscle of yellow perch at the Dome mine revealed a clear mine-related trend. The viscera of pearl dace also clearly demonstrated exposure to the Dome effluent for Ag, Cd, Cu, Se, Mo, Ni and Al. Viscera were responsive to exposure in blacknose dace but not small Atlantic salmon at Heath Steele. Caged wild fish were used to examine potential metal uptake at the Dome site and Heath Steele with little success.

The field results demonstrated that fish tissue metal levels generally reflected exposure to metal mine discharge. However, the data were sometimes variable between species, tissues sampled and metals analyzed. Fish muscle tissue is important to analyze where human health issues are of concern or interest, whereas other fish tissues may be more suitable for environmental health assessment.

The AETE Technical Evaluation (EVS 1998, Report #2.2.3) recommended that metal levels in fish should be measured but did not specify any particular tissues. The summary report on the 1997 field surveys (BEAK/Golder 1998c) recommended liver and muscle were the most appropriate tissues for a mine monitoring program due to practical considerations and consistency with previous studies. It was noted, however, that in some situations gill or kidney were also effective in measuring a response.

It is recommended that liver and muscle are suitable tools in a routine mine monitoring program. When small fish are used for study, whole viscera are also considered a suitable sampling medium for tissue metal levels. The use of gill or kidney for metal analysis may also be appropriate on a site specific basis to determine the biological availability of metals to fish.

5.2 Metallothionein

Metallothioneins (MT) are low molecular weight proteins that have a high binding affinity for Group IB and IIB metals. The synthesis of MT is increased in the presence of some metals notably Cd, Zn, Cu and occasionally Ag. Therefore, MT are often considered as a biomarker for exposure to these metals. Studies suggest they play a role in the regulation of essential metals such as Zn and Cu, and in the detoxification of nonessential metals such as Cd. Several elements have been shown to induce MT including Pb, Ni, As, Al, Fe and Mn.

The application of MT as a monitoring tool for evaluating the effects of mining in Canada was reviewed by Couillard and St-Cyr (1997; AETE Report #2.2.1) and is summarized in Toolbox Summary #7.2. In addition, MT levels were measured in fish tissues during all three AETE field programs (1995 -1997). The findings of those field studies are summarized in this section and in Table 5.1. Canadian scientists have been among the research pioneers for examining the use of MT as a monitoring tool (e.g. Klaverkamp and Duncan 1987). There is now an extensive body of scientific literature available on the use and application of MT in environmental monitoring studies and recent literature reviews are available (Roesijadi 1992; Stillman 1995) in addition to the AETE review mentioned above.

The use of biomarkers is based on the concept that contaminant-induced effects at the population, community or ecosystem level are preceded by biochemical reactions in individual organisms. Induction of MT in response to metal exposure has been observed in at least 20 different species of freshwater fish (Couillard and St-Cyr 1997).

The use of MT as a tool does require special attention to sample collection, handling and preservation, but these steps can be accommodated by experienced personnel. Samples collected in the field for MT analysis must be frozen on dry ice and transported to the laboratory frozen. Fish specimens must be kept alive until ready for dissection. Reliable analytical methods are known, but there is a need to standardize protocols for sample preparation, MT extraction and quantification. At present, MT analysis is not routinely performed by many private laboratories, although this expertise could be quickly developed. Under good storage conditions, MT levels should be stable for months to years, however, repeated thawing and freezing will affect results.

The 1995 AETE field program examined MT concentration in tissues of white sucker and northern pike from Exposure and Reference areas. There was no difference in tissue MT level between Exposure and Reference areas in 22 out of 23 comparisons. The one exception was MT in gill tissue of white sucker. Regression analysis did show a significant positive correlation between MT and metal levels in liver of adult white sucker and northern pike but not in other tissues.

The generally poor apparent sensitivity of MT as an indicator of metal exposure during the 1995 study may be a function of the absence of a clear metal gradient in water, and variability of the tissue metals data.

During the 1996 field program, fish were collected at 5 of the 7 mine sites and tissues analyzed for both metals and MT. In 2 of the 5 cases (Onaping/Levack and Gaspé), both MT and tissue metal levels were elevated in fish collected from the Exposure area relative to the Reference area (Table 5.1). At the Sullivan mine in British Columbia, neither tissue metal nor MT were different between the reference and exposure area, although waterborne metal levels were greater in the exposure area. At the Dome site, fish tissue metal levels were elevated in response to exposure to metals downstream of the discharge, but MT levels in the viscera of Pearl dace and Northern Redbelly dace were not different ($p > 0.05$) between areas. At Heath Steele, metal concentrations in juvenile Atlantic salmon were inconclusive and it was suggested that fish migration between the collection sites may have confounded the results.

Table 5.1 Summary of Fish Tissue, Metal and MT Results at Five Sites During the AETE Field Surveys

Year	Mine Site	Fish Species	Comments
1995	Val d'Or	White sucker Northern pike	Gill MT levels different in sucker but not in other tissues or species.
1996	Sullivan	Sculpin	Water metal levels elevated below mine, but no difference in tissue metals or MT
1996	Dome	Pearl dace N. Redbelly dace	Water and fish tissue metal levels elevated below mine but no difference in MT levels
1996	Onaping	White sucker	Metal levels elevated in liver and gill but not kidney. MT levels elevated in exposure area in all tissues
1996	Gaspé	Atlantic salmon	Fish metal and MT levels elevated in exposure area
1996	Heath Steele	Atlantic salmon	Fish metal and MT results inconclusive
1997	Heath Steele	Blacknose dace Atlantic salmon	MT response in blacknose dace and caged salmon, but not wild salmon
1997	Dome	Yellow perch Pearl dace	Tissue metal levels elevated downstream but no difference in MT levels
1997	Mattabi	White sucker Northern pike	Partial differences for both metal and MT

The 1997 results at the Dome site were similar to 1996, where fish tissue metal levels were elevated in the exposure area, but there was no difference in MT levels. At Heath Steele, the MT response was noted in viscera of blacknose dace and caged juvenile Atlantic salmon, but not in wild salmon. At Mattabi Mines, there was an MT response in gill and kidney of northern pike but not in liver; nor was there a response in any of the white sucker tissues. Of the 9 possible combinations of mine site and tissues surveyed in 1997, MT was partially effective at measuring a response in 4 of the situations, and did not demonstrate an effect in 5 cases. Overall, effects were more often demonstrated for metals than for MT (BEAK/Golder 1998c).

There was variability of the MT results introduced by the study design and biological variables. It must be noted, however, that the AETE studies reflected "real" mine situations. In these cases the limited effectiveness of the MT (i.e. absence of distinct spatial MT response to exposure) may be explained by some of the following confounding factors such as:

i) low metal bioavailability;
ii) absence of a marked gradient of contamination at some sites;
iii) intermittent effluent discharges at Dome site;
iv) history of fish exposure (mobility);
v) difference in conditions (physico-chemical characteristics) at reference sampling site; and
vi) confounding effects of other sources of contaminants.

Other biological factors may influence the results such as inter-fish variability (due to size, weight and age) and gut content (small fish were not subjected to a depuration phase). Many of these factors will require further research in order to include MT in future monitoring programs.

In summary, based on the results of the AETE field program, MT in fish may reflect exposure to some metals, but measuring metal levels directly in tissues appears to be a more reliable and cost effective means of measuring fish exposure to mine effluent.

Therefore, AETE does not recommend the use of metallothionein to measure the bioavailability of metals in a routine monitoring program.

6. IS THERE A MEASURABLE BIOLOGICAL RESPONSE?

The monitoring tools reviewed in this chapter focus on being able to detect a biological response in the receiving environment that is a result of exposure to mining effluent. Sublethal effluent toxicity tests are reviewed in this chapter because under the AETE program sublethal tests were evaluated as a tool for predicting downstream biological responses (Hypothesis 13). Sediment toxicity tests are also discussed here since the procedures use sediments collected from the receiving environment for toxicity testing. These differ from the acute toxicity tests discussed in Section 4.2.

The three major groups of organisms considered in this section include aquatic plants, benthic invertebrates and fish. The biological responses are considered along a range of biochemical, physiological, individual, population and community levels (Munkittrick and McCarty 1995). Figure 6.1 provides one framework to illustrate how stress responses are integrated at the individual, population and community level (from Munkittrick and Power 1990). Within each level of biological organization, responses may be considered as primary, secondary or tertiary.

Primary responses are very rapid and transitory, short-lived and generally reversible. Secondary responses are of longer duration than primary responses but generally also reversible. Tertiary responses are the least reversible and the longest lasting. Figure 6.1 illustrates how the stress responses are not simply translated along a continuum from the biochemical to community levels. Rather, movement between levels only occurs at well defined intersections or integration points where some responses are translated to the next level.

Recognition that not all responses are transferred to higher levels has significant implications for the selection of biological monitoring techniques (EVS 1998). It is important to understand the limitations of monitoring techniques since the choice of a particular tool at differing levels of complexity will directly influence the ability to detect and trace the response, to establish cause and effect, and to predict the consequences and ecological relevance. This latter point is perhaps the one issue that generates the most discussion among the various stakeholders involved in environmental monitoring programs.

Biological responses are sometimes viewed from a bottom-up (reductionist) approach or a top-down (holistic) approach (Figure 6.1). The bottom-up approach examines responses at the chemical or biochemical level. The advantage to this approach is that responses are generally rapid, allowing early detection of stressors. Biochemical responses are generally considered to have high specificity to the causal agent(s). A critical review of the available information, however, suggests that the number of highly specific biochemical indicators in aquatic systems may in fact be quite limited.

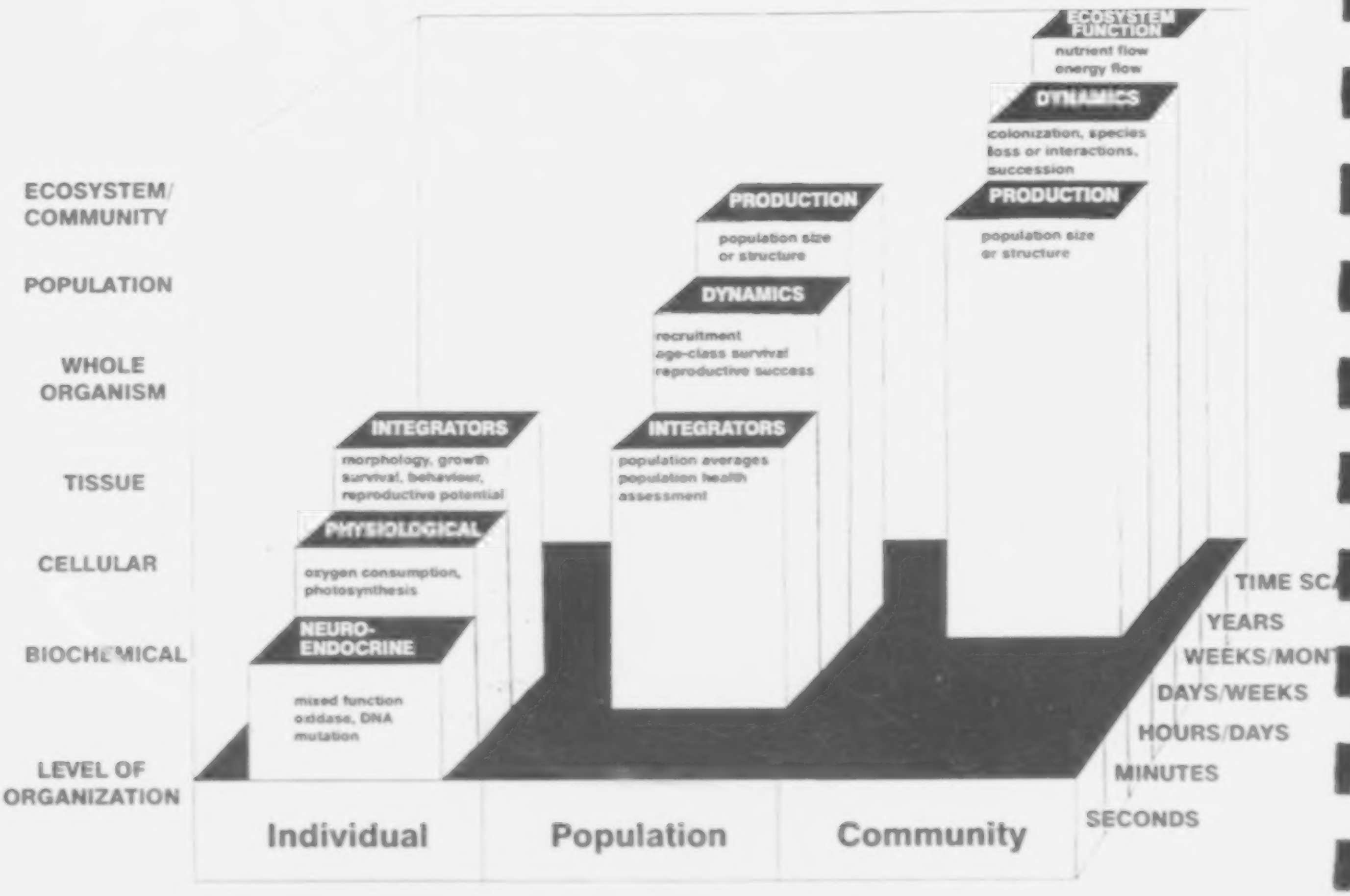

ECOSYSTEM FUNCTION
nutrient flow
energy flow
DYNAMICS
colonization, species
loss or interactions,
succession
ECOSYSTEM/
COMMUNITY
PRODUCTION
population size
or structure
PRODUCTION
population size
or structure
POPULATION
DYNAMICS
recruitment
age-class survival
reproductive success
WHOLE
ORGANISM
INTEGRATORS
morphology, growth
survival, behaviour,
reproductive potential
INTEGRATORS
population averages
population health
assessment
TISSUE
PHYSIOLOGICAL
oxygen consumption,
photosynthesis
CELLULAR
TIME SC
YEARS
NEURO-
ENDOCRINE
mixed function
oxidase, DNA
mutation
BIOCHEMICAL
WEEKS/MON
DAYS/WEEKS
HOURS/DAYS
MINUTES
LEVEL OF
ORGANIZATION
SECONDS
Individual
Population
Community

Figure 6.1 Impact Assessment Framework (Munkittrick and Power 1990)

The disadvantage to the bottom-up approach is that the consequences of specific biochemical responses within individual organisms are often poorly understood, and effects may be totally absent at the population or community level. In other words, there is no demonstrated continuum between the lower and higher levels of organization. As an example, MT seems to fall into this category given our current level of understanding. Biological responses are generally not considered ecologically relevant unless they are manifest at least at the individual level, and become increasingly relevant at the population or community level.

Addison (1996) developed a simple conceptual framework showing the relationship between specificity, ecological relevance and biological levels of organization (Figure 6.2). Community changes are highly ecologically relevant, but often cannot be related to specific causes. This is particularly true where there may be multiple effluent sources to a waterbody, animal migration is an issue, there are confounding habitat factors, or where natural seasonal or annual fluctuations are known to affect certain populations. Population or community responses may also not be obvious or measurable for time periods extending months or years depending on the organism's life cycle.

The appropriate level for biological monitoring has recently received considerable attention with regard to fisheries studies in Canada. Increasing attention has been given to what has been characterized as a middle-out approach (Munkittrick and McCarty 1995). This approach recognizes that the bottom-up and top-down methods start at opposite ends of the scale but both move toward the individual organism. Although there are many potential endpoints or responses that can be measured in individual organisms, generally only growth, reproduction and survival are likely to be directly transferred to population or community level responses.

The AETE program has aimed to take the environmental monitoring process beyond traditional programs by providing a framework for integrating and relating the tools and measured variables. The integration framework and methodology is described in Chapter 7. The material presented in Chapter 6 continues to lay the foundation for this approach by describing some of the tools that can be reliably used to measure biological responses in a mine monitoring program.

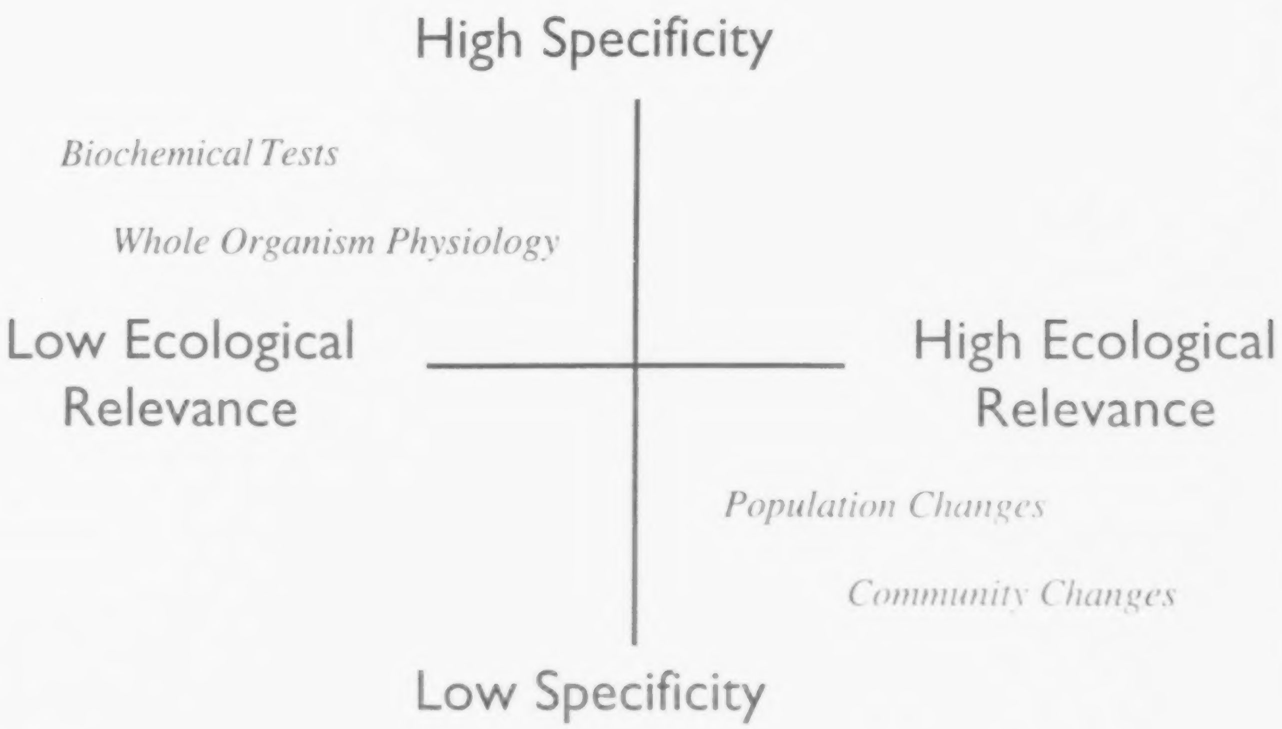

High Specificity
Biochemical Tests
Whole Organism Physiology
Low Ecological Relevance
High Ecological Relevance
Population Changes
Community Changes
Low Specificity

Figure 6.2 Ecological Relevance and Specificity of Biological Effects Measurements (from Addison 1996)

6.1 Sublethal Effluent Toxicity Tests

Sublethal tests have not been used as frequently as acute toxicity tests in regulatory programs in the past, however, their use is expected to increase. The terms "sublethal" and "chronic" are sometimes (incorrectly) interchanged. Most tests considered in this part of the AETE program are sublethal, that is, non-lethal endpoints such as growth and reproduction are measured. Mortality may occur during the test in which case survival is a legitimate endpoint to document. The term *chronic* is correctly applied to the test duration with respect to the life cycle of an organism.

Environment Canada toxicity test methods define chronic to mean occurring during a relatively long-term period of exposure, usually a significant portion (e.g. 10% or more) of the life span of the organism. The *Ceriodaphnia dubia* reproduction test would be considered a chronic test while the fathead minnow growth test is not.

This section describes the sensitivity and practical considerations of different sublethal tests for assessing the toxicity of mine effluents. The linkages between sublethal test results and in-stream biological responses (Hypothesis 13) are presented in Chapter 7.

A candidate list of thirteen sublethal toxicity tests was originally developed by the Toxicity Subcommittee for consideration by AETE (Table 6.1). The list reflects a diversity of organisms. The test endpoints also represented a range of levels of response from biochemical indicators to whole animals.

Table 6.1 List of Sublethal Tests Proposed for Evaluation in the AETE Program

General Type of Test	Specific Tests
Two tests for genotoxicity using bacteria.	Mutatox SOS Chromotest
Two biochemical tests:	Mixed function oxidases (MFO) in rainbow trout liver Metallothionein in trout liver
One performance test on bacteria	Microtox chronic
Three plant tests: one multi-cellular single species algae multi-species algae test	Duckweed (*Lemna*) growth *Selenastrum* growth Three algae species growth (*Microcystis* sp., *Selenastrum* sp., and *Nitischa* sp.)
Two invertebrate tests:	Nematode survival and growth *Ceriodaphnia* survival and reproduction
Three early life stages of fish:	Fathead minnow embryo-larval survival and teratogenicity Fathead larval survival and growth Rainbow trout embryo survival and growth

These original thirteen tests were critically reviewed by Sprague (1997; AETE Report #1.2.1) and discussed by the Toxicity Subcommittee. The candidate tests were evaluated on the basis of:

- Availability of standardized protocols
- Ecological relevance
- Technical procedures
- Cost and economy

Of these, relevance was considered the single most important criteria (weighted 40% of total score). **Subsequently, four of the candidate sublethal tests were screened out from further consideration. These included: SOS Chromotest, fathead minnow teratogenicity, mixed function oxidases (MFO) induction in trout liver and metallothionein in trout liver.** The remaining 9 tests were recommended for further testing and field validation.

In early 1996, a laboratory screening of the remaining 9 sublethal tests was undertaken. Eight effluents representing different mine types were sampled and subject to a battery of the 9 sublethal tests. The effluent samples were subdivided and submitted to three different toxicity testing laboratories in Canada (BAR Environmental Inc., Saskatchewan Research Council, Environment Canada). The results of all these tests are provided in AETE Report #1.2.2 (BAR 1997a). An overview description of each of these tests is provided in Appendix B in Toolbox Summaries #8.1 to 8.9.

The laboratory screening study (AETE Report #1.2.2) examined the results of the 9 toxicity tests with respect to:

1) sensitivity relative to each other;
2) relationship between effluent toxicity and effluent chemistry;
3) relative cost of each assay;
4) relevance; and
5) practicality.

Points were awarded for relevance if the test organism was native to Canada and if the protocol permitted the use of receiving water as dilution water. Practicality was based on the volume of effluent and/or receiving water required to perform the test.

Of interest to members of the AETE Toxicity Subcommittee was the potential influence of receiving water quality on test results when used as dilution water. Therefore, receiving water from the mine sites was used as control and dilution water in these toxicity tests and in the 1996 field program. In addition, a study on the potential toxicity of "Highly Mineralized Water (HMW)" was undertaken (see discussion below). For this screening study, site receiving water was from upstream or reference areas used as dilution water for *Ceriodaphnia dubia*, *Selenastrum capricornutum*, *Lemna minor*, fathead minnow, rainbow trout embryo and the algae multi-species phytoplankton tests.

Preliminary tests were undertaken to examine the effect of low ionic strength receiving water on test organisms. It was determined that growth or survival of fathead minnows or reproduction of *Ceriodaphnia* was not affected as long as the water hardness was > 3.9 and 5.5 mg/L, respectively. However, receiving water during the tests did influence the organisms with responses ranging from toxicity in fathead minnows, *Ceriodaphnia* and trout embryo test, to stimulation of growth of *Selenastrum* and increased reproduction of *Ceriodaphnia* in some cases.

Sensitivity

A simple comparison of the test results based on sensitivity produced four groups from most to least sensitive:

- *Selenastrum* and the multi-species phytoplankton test,
- *Lemna minor* and *Ceriodaphnia*,
- fathead minnow, and
- Microtox.

Sensitivity could not be evaluated in the nematode, Mutatox or rainbow trout embryo tests due to the nature of the test results or the frequency of invalid tests.

In general there were few correlations between effluent chemistry and toxicity. This may be the result of relatively small sample size (e.g. 8 effluents but not all showing a toxic response) and relatively high detection limits for the metal analysis. However, toxicity of effluents to *Selenastrum* was inversely correlated with total dissolved solids (TDS), conductivity and hardness.

Cost

Toxicity test costs were estimated by adding the cost of labour (testing, culture and QA/QC) and disposable materials. The calculation does not include the costs for overhead, administration or capital equipment. The cost estimates are not the actual amount that would be charged to perform the toxicity tests by a commercial laboratory, which might be 3 times the values below.

Based on this estimate, the toxicity tests fell into four general categories:

Test	Relative Cost
Selenastrum, Lemna	< $100.00
Microtox chronic, algae multi-species	$100.00 - $200.00
fathead minnow, *Ceriodaphnia*	$300.00 - $400.00
rainbow trout embryo	$700.00

Relevance

With the exception of Microtox (which uses a marine bacteria), most of the tests were considered relevant to the Canadian mining industry. Toxicity testing with the fathead minnow is restricted in Canada since it is not native to British Columbia, Newfoundland or the Northwest Territories and is not permitted in those areas.

Practicality

A second major consideration is the volume of effluent and dilution water required for a test. Again, the tests grouped themselves into four general categories:

Test	Total Volume required (L)
Microtox, algae multi-species, *Selenastrum*	< 1L each
Ceriodaphnia, Lemna	< 10L
fathead minnow	75L
rainbow trout embryo	295L

At the conclusion of the first mine effluent screening study, an additional four toxicity tests were removed from further consideration. These included the nematode test due to problems with the design and protocol; and the Mutatox test since the results were an "all or none" response. It was also decided to drop the Microtox chronic test due to low sensitivity and relevance. Although the phytoplankton multi-species test was shown to be sensitive, the test was dropped from further consideration since results were similar in sensitivity to the results of the *Selenastrum* test and the latter test has a published standardized test method protocol. The sensitivity of the trout embryo test could not be evaluated in this study since many tests were invalid as a result of poor gamete quality, but it was considered to have sufficient merit and potential to continue with further evaluation in subsequent studies. **The five remaining sublethal tests included: growth inhibition with *Lemna minor* and *Selenastrum capricornutum*; survival and reproduction of *Ceriodaphnia dubia;* growth and survival of larval fathead minnows, and the rainbow trout embryo survival test.**

Members of the AETE Toxicity Subcommittee agreed to examine the possibility that some natural surface waters may be toxic to test organisms due to watershed geology. Mines exist in geologically anomalous areas where elevated metal levels are common. Surficial mineralization and weathering of bedrock can lead to high background metal concentrations in the water and sediments. This matter was examined by collecting a sample of Highly Mineralized Water (HMW) from an upstream location at one mining site and testing the water with the suite of 5 remaining sublethal toxicity tests.

For the purpose of the AETE program, HMW was considered to include water coming into contact with naturally mineralized zones and containing elevated levels of metals and major ions, especially sulphur. Although this criteria may be met at other locations, only one sample of HMW from Labrador was collected as part of the program. The results of the toxicity tests are described in AETE Report #1.2.4 (B.A.R. 1997b). A second objective of the HMW test was to determine, if the sample was toxic, whether *Ceriodaphnia* and fathead minnows could be acclimated to the HMW water.

The Labrador natural HMW contained elevated concentrations of copper and nickel at 430 and 1120 µg/L, respectively. The sample was slightly acidic, pH 5.9, with low alkalinity of 2 mg/L. The sample displayed considerable toxicity to all test organisms with most animals dying during the test. Similarly, slow acclimation to HMW using fathead minnows and *Ceriodaphnia* was unsuccessful. It is suggested that a larger survey be undertaken throughout Canada to identify the scale of the issue and degree of variability in background conditions.

The 5 remaining sublethal tests were used in the 1996 Field Survey which considered 7 candidate mine sites across Canada. The primary purpose of the 1996 field work was to identify 3 - 5 mine sites where the AETE tools and their hypotheses could be tested during the 1997 field program. As part of the 1996 field program, effluents were collected at each of the 7 sites (involving 8 effluent discharge points) in the fall of 1996 and tested for sublethal toxicity. The study objectives included:

- characterize the toxicity of the 8 mine effluents,
- further evaluate relative sensitivity of the tests,
- determine if natural receiving waters cause toxicity,

- evaluate acclimation procedures for fathead minnow and *Ceriodaphnia*, and
- gain further practical experience with the five toxicity tests.

The results of the toxicity tests are summarized in each of the 1996 field reports (EVS 1996a, 1996b, 1996c; ESP 1996a, 1996b; JWEL 1996a, 1996b).

All effluents exhibited some toxicity although they were generally not very toxic. The IC25 results range from 5.1 to >100% effluent by volume (Table 6.2). The tests could be divided into two groups based on sensitivity with *Selenastrum*, *Lemna* and *Ceriodaphnia* generally being the most sensitive. The fathead minnow was consistently the least sensitive of the tests. These results supported the findings of the earlier mine effluent screening study.

Table 6.2 Summary of 1996 Results (IC25) and Relative Sensitivity for Sublethal Toxicity Tests. Results are expressed as % v/v effluent.

Mine	*Selenastrum* growth	*Lemna minor* growth	*Ceriodaphnia* reproduction	Fathead minnow growth/survival
A	>100	31.8	79.4	>100
B	23.3	47.3	19.0	23.0
C	30.8	14.2	80.7	>100
D	>100	21.7	>100	>100
E	22.2	27.2	12.6	>100
F	5.1	18.3	33.5	64.4
G	47.6	37.0	67.0	82.1
Sensitivity	2	1.9	2.1	3.0

Samples of receiving water from each mine site were submitted to the toxicity testing laboratory prior to effluent sampling to screen for potential toxicity. Natural receiving water from two of the sites (Sullivan, Gaspé) caused toxicity to fathead minnows and *Ceriodaphnia*. Therefore, these test organisms were acclimated to water from these two sites before effluent testing was initiated using the receiving water as dilution water. Acclimation was successful in increasing survival and effluent test results were then valid.

The responses of nonacclimated organisms to receiving waters were variable ranging from toxicity to stimulation. Growth of *Selenastrum* was stimulated in water from 5 of the 7 sites, and *Lemna* growth was stimulated in three of the waters. Prolonged storage time of the receiving water may have affected the observed responses.

Considerable practical experience using these different tests was gained during the program. Some initial difficulties were noted using the Saskatchewan Research Council test protocol for *Lemna minor* toxicity testing. The problems were encountered with contamination by natural algae populations. Culturing under aseptic conditions should eliminate these problems.

Four of the seven rainbow trout embryo tests were considered invalid primarily due to poor gamete quality from the hatchery resulting in poor fertilization success in both receiving water and laboratory water tests. The cause of low fertilization was likely poor quality eggs and/or milt used for the tests, and was similar to problems experienced during the earlier laboratory screening test program. Where the trout embryo tests were valid, the results showed similar sensitivity as fathead minnows tested on the same mine effluent. Another practical consideration of using the trout embryo test was the very large volume of receiving water required for daily renewal of the solutions. Approximately 295L (eg. 15x 20L pails) of water are required. This represents significant effort and cost to collect and ship such large quantities of water from remote mine sites. (Note: following AETE the protocol was revised to use much lower volumes of water).

In summary, due to problems with poor gamete quality from hatcheries and for practical reasons, the rainbow trout embryo test is not recommended as a routine tool for assessing the aquatic effects of mines.

Effluent for sublethal toxicity testing was collected from three mine sites (Dome, Myra Falls and Heath Steele) on three separate occasions during the 1997 field survey. Samples were tested using the four remaining sublethal tests (fathead minnow, *Selenastrum, Lemna, Ceriodaphnia*). All samples elicited toxic responses with *Lemna*, *Selenastrum* and *Ceriodaphnia* generally being the most sensitive. Fathead minnows were generally the least sensitive. Figure 6.3 provides the mean IC25 results from the three Myra Falls bioassays which illustrates this trend in sensitivity.

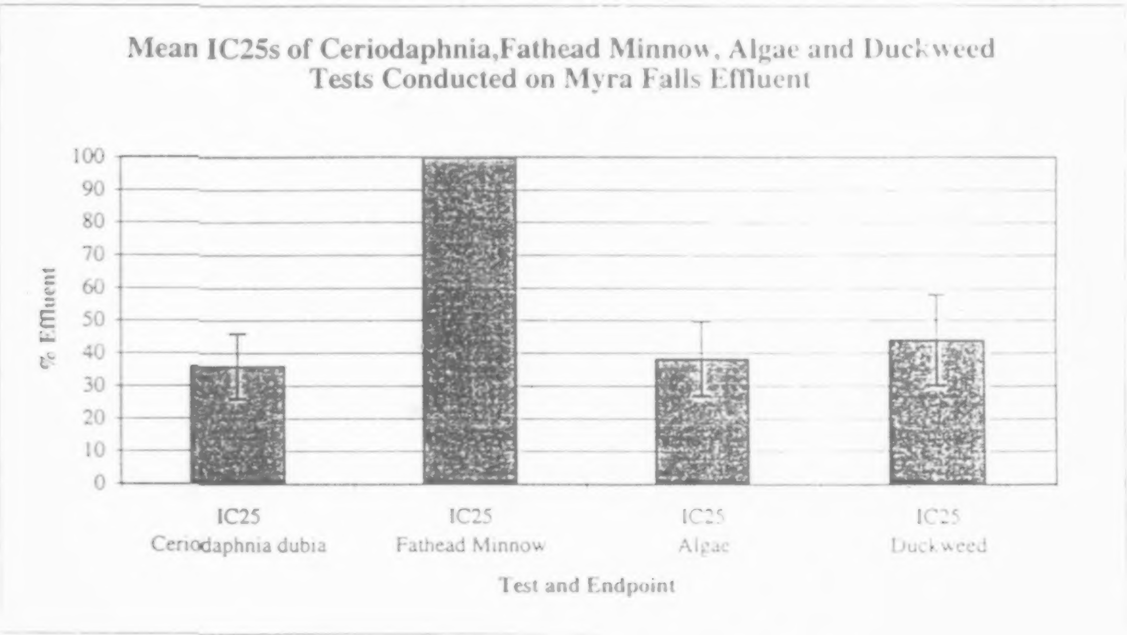


Figure 6.3 Mean Toxicity Results (± 1S.E.) for Four Sublethal Test Species at Myra Falls, June, August, November, 1997.

In summary, all four remaining sublethal toxicity tests proved effective in their ability to detect an effect in mine effluent. The tests have demonstrated sensitivity, are commercially available with standardized protocols and are ecologically relevant.

Therefore, the following four tests were found suitable as candidate tools for routine sublethal testing of mining effluents: Fathead minnow, *Selenastrum*, *Lemna minor* and *Ceriodaphnia*.

6.2 Sediment Toxicity Tests

Sediment toxicity tests are commonly used to evaluate potential contamination in marine and freshwater environments. These tests provide a direct method to determine chemical availability and can be used in conjunction with chemical measurement data. The AETE program evaluated four different sediment toxicity tests to address their relative ability to detect exposure to a mine discharge. The comparisons were also used to test Hypothesis 1: *The strength of the relationship between sediment toxicity responses and any exposure indicator is not influenced by the use of different sediment toxicity tests or combination of toxicity tests.* The tests considered were:

- growth and survival of the amphipod *Hyalella azteca*
- survival and reproduction of the oligochaete *Tubifex tubifex*
- Microtox solid phase test
- survival and growth of the freshwater midge *Chironomus riparius*

These tests were applied during the 1995 and 1997 field programs. No literature review was undertaken as part of the AETE evaluation. The general protocols and methods for the sediment toxicity tests are summarized in Toolbox Summaries #9.1 to 9.4.

The selection of appropriate reference areas is one of the critical components for application of sediment toxicity testing. The primary purpose of reference sediments is to provide a geochemically similar substrate to test (exposure) sediments to measure relative effects which are not contaminant related. Metal bioavailability, and hence sediment toxicity, is influenced by several physical characteristics of the sediment. Grain size, ammonia, sulphur, and total organic content are among the most important variables that should be considered when choosing reference sediments.

Test protocols are available for each of the sediment tests used. The species *Hyalella azteca* is a freshwater amphipod that feeds on surface detritus. Test organisms are counted for survival and growth (weight gain/loss) is recorded (Environment Canada 1996a) after the 14 day exposure period. The *Tubifex* test uses mature (> 8 week old) animals which are exposed to test sediments for 28 days. At the conclusion of the test, the sample is sieved and the number of surviving adults are counted. As well, the number of offspring produced are counted as a measure of reproductive success (ASTM 1992). In the *Chironomus* test, first or second instar organisms are exposed to test or reference sediments for a 10 day period (Environment Canada 1996b). At the test conclusion, the sample is sieved and animals counted (survival) and weighed (growth). Results for all of these tests are sometimes expressed as a proportion (%) of response from the reference sediments. Both *Tubifex* and chironomids are burrowing organisms.

The Microtox test is a commonly used rapid screening bioassay that uses a luminescent marine bacteria. In the solid phase (soil or sediments) test, the bacteria are allowed to come in contact with a suspension of the test sample. The results are expressed as percent reduction of luminescence relative to the control or reference sample after a 20 minute exposure period.

During the 1995 field program, sediment samples (approximately 10L per site) were collected from 14 different stations including four reference areas. Survival of *Hyalella* appeared to be the most sensitive test. However, reproductive performance of *Tubifex* showed the best graded response to exposure and showed less between site variability (BEAK 1996). The two organisms display generally different sensitivities to environmental contaminants. *Tubifex* are reasonably tolerant to stress, therefore, sublethal responses such as reproduction can be measured. In comparison, *Hyalella* is more sensitive, and growth may be an irrelevant parameter to measure if there is little survival.

The Microtox test only displayed a significant response for the most contaminated sediments but was not sensitive to an environmental gradient of exposure. Although it was considered a good screening indicator of toxicity the advantages of the test were offset by reduced sensitivity. **Therefore, the Microtox test was not recommended for further evaluation in the AETE program.**

During the 1997 field program, sediment effects were examined using the *Hyalella*, *Tubifex* and *Chironomus* toxicity tests. The three tests were applied at three of the four sites; Myra Falls, Dome and Mattabi. Suitable quantities of depositional sediments were not available at Heath Steele in New Brunswick. Sediments at Mattabi did not elicit any toxic responses, therefore, the relative sensitivity of the tests are based on results from two sites.

The effectiveness of sediment toxicity as an indicator of a measurable response is demonstrated by the difference in toxicity between reference and exposure areas and/or the occurrence of trends within the exposure area (near-field and far-field).

Sediment toxicity reflecting exposure to mine discharge was evident in mortality and growth impairment in *Hyalella* at Dome and Myra Falls but not at Mattabi (Table 6.3). The *Chironomus* tests were only effective at Myra Falls. The magnitude of the reference-exposure difference was greatest for *Hyalella* and *Chironomus* at Myra Falls indicating that these tests were more effective than the *Tubifex* test. The *Tubifex* test was not effective at demonstrating an effect at either the Dome or Mattabi mines.

Table 6.3 Comparison of the Effectiveness of *Hyalella azteca, Chironomus riparius* and *Tubifex tubifex* Toxicity Monitoring Tools (Hypothesis H1)

	Sediment Toxicity Tools			
Mine Site	***Hyalella azteca***	***Chironomus riparius***	***Tubifex tubifex***	**Comments**
Dome Mine	√	X	X	No mine-related response in *Tubifex* or *Chironomus*. Mine-related trend in *Hyalella* mortality and growth.
Heath Steele	NT	NT	NT	
Mattabi Mine	X	X	X	All tests showed no significant reference-exposure differences or trends in the exposure area, thus, no discernible difference in effectiveness. Area effects were evident for sublethal but unrelated to exposure were evident for sublethal responses in *Chironomus* and *Tubifex*.
Myra Falls	√*	√*	√	Mortality increased with exposure for *Hyalella* and *Chironomus* tests, but not for *Tubifex*. *Tubifex* responded in terms of reproductive effects.

NT - Not tested.
√ - Effect demonstrated.
X - Effect not demonstrated.
P - Effect partially demonstrated.
* - More effective at demonstrating an effect.

In general the *Hyalella* test was most effective at demonstrating a mine effect. The *Chironomus* test showed similar sensitivity at the one site, and also demonstrated a good relationship with sediment chemistry (see Hypothesis 10, Section 7). The costs for *Hyalella* and *Chironmus* tests are similar (about $600 each) while *Tubifex* testing is not widely available and the cost is expected to be somewhat higher (ca. $800) since the test duration is longer.

In summary, AETE recommends that both the *Hyalella* and *Chironomus* tests are suitable sediment toxicity tests for routine monitoring purposes. The *Tubifex* test also demonstrates potential and may be considered as an alternate species or used in a suite of toxicity tests on a site-specific basis.

6.3 Aquatic Plants

6.3.1 Community Structure

Species composition and community structure of macrophytes, periphyton and plankton as monitoring tools were reviewed by St-Cyr *et al.* (1997) for the AETE program (Report #2.3.2) and are summarized in Toolbox Summaries #10.1 to 10.3. Community structure of periphyton was examined at Heath Steele during the 1997 field program but not as a component of any of the other field surveys.

Measures in biomonitoring studies include approximate counts of species presence/absence, abundance of certain indicator species as well as species diversity and richness (Small *et al.* 1996). Examination of macrophyte communities adjacent to point source discharges reveals some common responses including:

- a decline in species number,

- increased density of a few tolerant species, and
- gradual changes in the community composition along a gradient of recovery.

The literature review conducted for AETE (St-Cyr *et al.* 1997) suggested that macrophyte species composition and abundance is a potential tool for evaluating the impacts of mining activities on receiving environments. However, this tool was not evaluated in the field program and a clear sampling and interpretation protocol has not been developed.

Phytoplankton can display a variety of responses to metal exposure including changes in species composition, density, biomass and production. Density and biomass of phytoplankton may decrease or actually increase due to reduced grazing pressure in contaminated ecosystems. Biomass may also increase as a few remaining tolerant species dominate the community. Phytoplankton communities also undergo substantial natural seasonal, and in some cases diurnal, fluctuations which makes data interpretation difficult.

Sorting and identifying plankton samples is a time consuming process, and there are relatively few trained taxonomists available should species composition be considered a suitable monitoring tool. As an alternative to detailed taxonomy some techniques have been developed to examine size distribution of particles in the phytoplankton community (Cairns *et al.* 1993). However, this methodology has not received field validation and is not suitable for routine monitoring.

The use of periphyton community structure was reviewed by St-Cyr *et al.* (1997; AETE Report #2.3.2) and summarized in Toolbox Summary #10.2. The taxonomy of periphyton communities was also examined at Heath Steele as part of the 1997 field evaluation. The three main approaches to using periphyton in monitoring programs include:

- Indicator species - presence and absence of key species
- Mathematical indices - such as biotic index, diversity, species richness
- Functional responses - including metabolism, photosynthesis, biomass

Samples of periphyton were collected for taxonomic evaluation from each of the eight study reaches at Heath Steele in 1997. All samples were rich in algal species and variable in terms of biomass. There were no spatial trends in biomass or number of taxa apparent between the exposure and reference reaches that could be related to effluent gradients (BEAK 1998b).

The major constraint with using periphyton as an indicator is to differentiate natural variability from changes induced by metal contamination. Some researchers have used artificial substrates to address this problem. However, St-Cyr *et al.* (1997) suggest that standardized protocols and further research on periphyton responses to metal exposure are required before these are useful tools in routine monitoring programs.

The AETE does not recommend using aquatic plant community structure as a routine monitoring tool.

6.3.2 Biochemical Indices

The use of biochemical indicators in aquatic plants was reviewed for AETE by St-Cyr *et al.* (1997). A review of the techniques is provided in Toolbox Summary #10.4. These methods were not part of any of the AETE field evaluations.

The following general groups of biochemical indicators have been studied in aquatic plants:

Macrophytes: i. Phytochelatins, ii. Enzymes
Phytoplankton: i.Phytochelatins, ii. Enzymes, iii. Pigments

Phytochelatins are metal-binding proteins found in the plant kingdom. They can be considered the functional equivalent of metallothioneins in animals but are chemically different. This group of proteins has been studied extensively in terrestrial ecosystems (Gawel *et al.* 1996) and has been reported in aquatic macrophytes but not widely studied. Phytochelatins have been induced in plants grown in contaminated soils or media and may play a role in metal detoxification in plants. Phytochelatins have also been observed in phytoplankton, but to date only marine species have been examined. This technique has some potential as an indicator of metal exposure, but it would be premature to recommend it as a routine monitoring tool.

Accumulation of phototoxic levels of metals can result in either inhibition or induction of activity of enzymes involved with various metabolic processes. Under metal stress the enzyme peroxidase (POD) is induced particularly in higher plants, and holds some potential as an indicator of sublethal stress in a plant. In phytoplankton, the enzyme alkaline phosphatase (APHA) was inhibited by exposure to Cu in laboratory cultures. Enzyme activity is also highly correlated to phosphorous nutritional status of the algae. Further research is required to identify other factors that might also affect enzyme activity under field conditions, and to correlate responses to metal exposure before they can be considered a useful monitoring tool.

Metals are known to inhibit certain pathways involved with photosynthetic activity. As a result the pigment status of phytoplankton is altered due to different ratios of chlorophyll and carotenoids. It has been proposed that pigment analysis of phytoplankton by HPLC (High Performance Liquid Chromatography) might provide a method to evaluate exposure to metals, as well as shifts in taxonomic structure. However, their use as routine monitoring tools is not recommended because a) there is a lack of clear protocols required, b) the relationship between response and metal exposure is not well documented, and finally, c) the link between the biochemical indicator and more relevant ecological responses has not been demonstrated.

The AETE program does not recommend using plant biochemical indices as routine monitoring tools.

6.4 Benthic Invertebrates

The use of benthos as a tool in environmental assessments is well established. Benthic invertebrates are sedentary, reflect local conditions, and can therefore be used effectively to evaluate point-source impacts. They are also short lived and thus respond relatively quickly to changes in ambient chemistry and physical habitat. Monitoring with benthic invertebrates can involve characterization of community, population and individual endpoints. Typical assessments with benthos involve some characterization of the whole community, therefore the AETE program examined several factors that might influence such characterizations. Although population-level benthic endpoints are rarely encountered in environmental monitoring, the potential use of fitness parameters for endemic organisms and of transplanted bivalve molluscs was explored as part of the program.

6.4.1 Population Fitness Parameters

The potential use of benthic population fitness parameters as monitoring tools was discussed by Feltmate and Fraser (1998; AETE Report #2.1.5). They are described in Toolbox Summary #11.2. Fitness parameters were not evaluated in any AETE field trials except for chironomid mouth deformities in 1997.

Impacts on fitness parameters are generally evaluated with dominant or key taxa indigenous to a receiving system. One advantage to working with fitness parameters is that the study is limited to only a few taxa: all other taxa need not be processed. Fitness parameters that have been used in assessments include (1) density, (2) size, (3) condition, (4) fecundity, (5) adult emergence, (6) distributional (behaviour) changes, (7) morphological deformities, and (8) fluctuating asymmetry. Density, size, fluctuating asymmetry and condition can be determined with little effort beyond the typical benthic study. In contrast, fecundity, emergence, behaviour and morphological deformities require either specialized expertise or equipment and could be prohibitively costly to apply.

The eight fitness parameters reviewed are at an elementary stage of investigation and no standardized protocols are available. Morphological deformities have been used to evaluate the effects of chemically altered sites, but have rarely been used to assess the effects of metal-mining effluents. In the 1997 surveys, there was no relation between exposure to mine effluent and occurrence of chironomid mouth deformities.

Based on these results, benthic fitness parameters are not recommended as tools for routine monitoring.

6.4.2 Bivalve Mollusc Growth

A Technical Evaluation of mollusc growth was undertaken by Salazar (1997; AETE Report #2.3.1). The author proposed the use of transplanted (caged) bivalve molluscs to examine the presence of effects. Growth is one sublethal response can exhibit a dose-response relationship. For this reason, correlations between growth reductions and exposure can be used to develop an argument for a mine-effluent-related effect. In addition, growth represents the integration of all internal biological processes. See also Toolbox Summary #6.1.

The main advantage to the use of transplanted and caged bivalves was the potential for rigorous experimental control. Because bivalves can be caged along a gradient of exposure, it is possible to establish dose-response relationships and increase the confidence to infer cause-effect. Also, replication with caged mussels can be high to improve statistical power of the experiment. Caged bivalve experiments were not conducted in any of the AETE field programs.

Growth studies with caged bivalves have been conducted in marine, estuarine and freshwater environments using both indigenous and exotic species. However, protocols for conducting tests with bivalves have not been standardized, and results are variable depending on the season, species and habitat.

Therefore, AETE does not recommend that caged bivalve growth experiments are suitable for routine monitoring programs.

6.4.3 Benthic Community Composition

Assessments with benthic invertebrates typically involve some characterization of the benthic community found at a site. Differences in composition between a test site and a group of reference sites are then used as evidence that an impact has occurred. Since different collection and processing methods have the potential to give different characterizations of benthic community composition, several studies were commissioned by AETE to explore the issue. The first report was by Golder Associates Ltd. (1995; AETE Report #2.1.1) who conducted a review of artificial substrates for the collection of benthos. In 1995, BEAK (1996; AETE Report #4.1.1) conducted field trials to evaluate a variety of methodological factors. Finally, Taylor (1997; AETE Report #2.1.2) wrote a review to establish the suite of methods that would most reliably detect effects when they occurred. AETE also recognized that the way in which the benthic community data were summarized would potentially influence the validity of assessments. Consequently, an evaluation of methods for analysis and interpretation of benthic invertebrate communities was conducted by Taylor and Bailey (1997; AETE Report #2.1.3). The review by Taylor (1997) also addressed some issues related to data analysis.

Field and Laboratory Methods

The report by Golder Associates Ltd. (1995) examined the role of rock-filled baskets, Beak trays, rock-filled trays and multiplate samplers for collecting benthos (Toolbox Summary #11.4). Artificial substrates are generally unnecessary in shallow streams and rivers with cobble or gravel substrate, since it is easy to obtain natural substrates in these habitats. Rather, artificial substrates are potentially useful to sample those habitats in which it is difficult to obtain natural substrates (e.g., rivers with torrential currents). The main advantages of sampling with artificial substrates are that they (1) can be used to sample difficult habitat, (2) provide greater flexibility for study designs; (3) reduce variance in organism densities, and (4) collect a greater variety of organisms (also increasing statistical power).

In contrast, the use of artificial substrates is limited because they do not (1) characterize the natural bottom fauna, (2) indicate habitat conditions other than water quality, (3) estimate the availability of food organisms, or (4) integrate long-term effects of pollution. Artificial substrates also require two visits to a site and are prone to loss. If they are deployed during extreme conditions, they may also overestimate the effect occurring on fauna in natural substrates. In contrast, if deployed during more favourable water quality conditions, they will be less likely to demonstrate effects than natural substrate.

AETE does not recommend artificial substrates for routine monitoring but their use may be considered where suitable natural substrates are not present or other viable alternatives are not available.

The likelihood of detecting impacts resulting from metal mine discharges is increased if benthos are identified to lowest practical levels (i.e., genus/species), if mesh size for washing samples is kept fine (200-250 µm), and if the number of samples can be increased. Identification to genus/species is required because higher levels of taxonomy often result in species with contrasting tolerances being lumped together (Barton, 1996).

The "*Technical Evaluation on Optimization of Methods for Benthic Invertebrate Biomonitoring*" (Taylor and Mazier, 1997) provided a review and recommendations on mesh size (effects of different sieve meshes on sample integrity or bias, effects of changing sieve mesh size) and taxonomic resolution (identifications to species, genus, family, etc., cost-effectiveness of more detailed identifications, effects of mixed resolution).

Samples washed with fine mesh do take longer to process, and there are usually many taxa represented by immature individuals that are difficult to identify to genus/species. These factors increase sample processing costs. In other programs, 500-µm mesh is standard because samples take less time to process and because there are fewer unidentifiable immature forms.

The 1995 Pilot Study in Val d'Or briefly looked at a comparison of the sensitivity of small and large mesh sizes. The data suggested that smaller mesh sizes were slightly more sensitive. However, the data were insufficient (weak gradient of contamination) to make conclusions and recommendations on the cost-effectiveness and sensitivity of the different mesh size.

The 1997 field program did not evaluate various benthic processing procedures. Samples were processed using 250 µm mesh size and identification to Lowest Practical Level to ensure the highest sensitivity and reliable data to detect low effects, for testing hypotheses and for applying the Sediment Quality Triad. Additional evaluation of the data sets has been undertaken (BEAK/CANMET 1999, in preparation) to come to a definitive conclusion on sensitivity and cost-effectiveness of different mesh sizes and taxonomic identification for detecting mine-related effects and routine monitoring.

The size and number of benthic samples can be optimized to increase the likelihood of detecting effects. Rather than collect a few large samples, it is more cost-effective to collect a larger number of smaller samples. If possible, the use of corers or T-samplers that collect substrate from a relatively small area is recommended.

Study designs currently in use include the classic upstream-downstream comparison described fully in Green (1979). With this design, usually a single upstream reference site is used as a control to test for impacts at downstream "impacted" sites. Within each of the reference and impacted sites, multiple samples are collected and processed. This is the current design that is part of the federal EEM program for the pulp and paper sector (Environment Canada, 1998). This design is perceived by some as flawed because having only a single upstream reference site does not fully characterize the extent of the natural background variation in community composition, regardless of the number of samples collected in each location. As a result, if a difference between upstream and downstream locations is detected, it may be erroneously concluded that the difference is ecologically meaningful when it is not. In addition, if there is a difference in composition between the single upstream reference site and the downstream impacted sites, there is no guarantee that the sites were not naturally different. To improve on the design, benthos from a large number of "regional"-reference sites can be characterized to generate a better understanding of natural variation and therefore of the ecological relevance of any effects on benthos at downstream locations. Incorporating multiple reference sites into a study can obviously be costly, so there is a trade-off between costs and the probability of erroneously declaring a site impacted.

Rapid bioassessments (Toolbox Summary #11.5) with benthos are not appropriate for direct inclusion in monitoring programs for mining. Rapid bioassessments generally use less rigorous methodologies (coarse mesh, field identifications to coarse levels, minimal within-site replication). As such, the likelihood that they will detect anything other than gross effects is low. Rapid bioassessments do, however, have a role as screening tools (Plafkin *et al.* 1989). Because they are less intensive, a larger number of sites can be assessed, thus potentially improving our understanding of the spatial extent of effects.

For routine monitoring programs, rapid bioassessment procedures are not recommended.

QA/QC are also essential elements in any field or laboratory program. Prior to conducting a study, the QA/QC program should incorporate an appropriate study design with appropriate sampling procedures. QA for field programs should include (1) standard operating procedures for equipment and instrument maintenance and calibration, and sample collection and preservation, (2) chain of custody forms, (3) appropriate shipping and storage instructions, (4) trained staff, and (5) routine checks on the performance of field equipment. In the laboratory, QA can be provided if (1) there are well documented procedures for receiving and storing samples, (2) instruments are calibrated and maintained, and (3) appropriate methods are used for sorting and identification. Quality control can be established by conducting routine checks on sample sorting efficiency, the effects of sub-sampling and taxonomic accuracy.

Data Analysis and Interpretation

As with different field and laboratory methods, different approaches to data analysis and interpretation have the potential to lead to different conclusions. The objective of the report by Taylor and Bailey (1997) was to recommend analytical approaches that are valid, effective and ecologically relevant. The best analytical methods are those that derive the most useful information and provide the greatest sensitivity with lowest cost (Taylor and Bailey, 1997).

The use of hypothesis testing procedures (analysis of variance) is the preferred approach to establishing the likelihood that an effect had occurred. Multivariate clustering and ordination methods were discussed as being useful to portray similarities in composition among stations, but in contrast to advice from other authorities (Green, 1979), these methods were considered inappropriate for testing hypotheses.

The use of descriptors, such as total abundance and species richness, was recommended because they have a long history of use and are reasonably descriptive. Similarity indices were also recommended because they summarize the overall differences in composition, and because they require no preconceived assumptions about the nature of the impact.

A weight-of-evidence approach was strongly recommended by Taylor and Bailey (1997). The weight-of-evidence approach would use the results from analysis of variance on a number of taxa and community variables. Concordance among a number of community descriptors, that would *a priori* be hypothesized to respond to mining effluent, provides much more compelling evidence that an observed effect is or is not mine related. Increasing the number of descriptors increases the odds of detecting an erroneous difference. Multivariate analysis of variance is one method that can deal with the problems associated with interpreting several variables.

Quality of interpretation can be assured through appropriate data management that includes a data validation procedure, selection of appropriate statistical procedures, and ecological interpretation. Prior to any analysis, data should be checked to ensure that they meet the assumptions required by the underlying models, while the effects of missing values or outliers on interpretations should be established (BEAK 1998). If the classic upstream-downstream study design is used, one should be cautious when interpreting statistically significant differences in benthic community composition. Statistical differences with such designs can be achieved simply by increasing sample size, thus the ecological relevance of such effects can be challenged. To establish the ecological relevance of effects, the benthic indices from the affected location can be compared with the normal range of variation in benthic community indices from regional-reference locations (Kilgour *et al.* 1998; BEAK 1998).

Benthic surveys were an integral part of all three years of AETE field programs. Benthic invertebrates were used at some sites in 1997 to test Hypothesis 6: *There is no environmental effect in observed fish (or benthic) community structure.*

When considered individually, benthic community health indicators such as total density, number of taxa, EPT index (Ephemeroptera-Plecoptera-Trichoptera) and abundance of indicator taxa varied in their effectiveness between mine sites (Table 6.4, 1997 results only).

As shown in Table 6.4, the use of measures such as only the number of taxa and total density may not be adequate to distinguish mine-related effects. At sites where impacts may be slight to moderate, the sensitive taxa are numerically replaced by more tolerant taxa such that the area still maintains its carrying capacity with respect to numbers of individuals and taxa. This supports the need that many community descriptors and key indicator taxa should be monitored.

Table 6.4. **Summary of Effectiveness Rankings for Benthic Community Health Indicators**

	Benthic Invertebrate Tools				
Mine Site	**Total Benthic Density**	**No. of Taxa**	**EPT Index**	**Abundance of Indicator Taxa**	**Comments**
Dome Mine	√	√	√	√	Benthic indices such as number of taxa, density, EPT index (generic level) and indicator taxa all showed significant mine-related trends.
Heath Steele	X	P	√	√	Number of benthic taxa showed no linear trend in exposure zone. Number of EPT taxa was reduced with exposure. No spatial trends evident in total density. *Rheocricotopus* dominance showed a linear trend in exposure area and an exposure/reference mean difference. Orthoclad dominance showed a trend in the exposure area.
Mattabi Mine	√	√	NT	√	Significant decrease in exposure area, and exposure area trends in density, number of taxa and indicator taxa.
Myra Falls: • Stream • Lake	 X X	 X X	 √ NT	 √ √	Key indicator taxa abundances responded to exposure, including EPT index, Ephemerellidae, *Cricotopus* + *Orthocladius* and total Chironomid abundances in creek, *Pisidium* and harpacticoid abundances in Buttle Lake.

NT - Not tested
√ - Effect demonstrated
X - Effect not demonstrated
P - Effect partially demonstrated

Benthic monitoring tools were effective for demonstrating mine-related effects. **AETE recommends that benthic invertebrate community composition is a suitable tool for a routine monitoring program.**

6.5 Fisheries

6.5.1 Biochemical Indicators

All fish body functions are controlled by various hormones, enzymes, and proteins, many of which have been used as potential indicators of biological responses. In this context they are potential indicators of biological responses to stress induced as a result of exposure to mining effluent. The use of biochemical indicators in fish was reviewed for the AETE program by EVS Consultants (1998; Ref. # 2.2.3) and is summarized in Toolbox Summary #12.1. Biochemical indicators were not evaluated in any of the AETE field programs.

The biochemical measure in fish showing the highest specificity to a metal is inhibition of delta Aminolevulinic Acid Dehydratase (ALAD) by lead. This enzyme governs hemoglobin synthesis. A clear relationship between lead exposure and ALAD activity has been demonstrated. However, the relationship between fish performance and ALAD activity is not clear especially at low lead levels, suggesting ALAD may be a better indicator of exposure than as a biological response.

Other enzymes and protein indicators include cholinesterase enzymes, hepatic mixed function oxidases (MFOs), brain monoamine oxidase (MAO), ATPase activity, leucine Aminonapthyl-amidase (LAN) activity, sorbitol dehydrogenase (SSDH), and transaminsase enzymes. Schlenk *et al.* (1996) recently compared several hepatic biomarkers with a series of whole animal, population and community measures of fish health. Out of over 100 potential correlations, only two were significant.

Exposure to metals and low pH conditions associated with acid mine drainage can alter blood and whole body ionic and osmotic regulation in fish (e.g. Folmar 1993). Concentrations of blood Na, Cl, K and Ca ions generally decrease in fish exposed to elevated acid and metal levels. In particular, Na loss has been the most extensively validated in the field and may hold some potential for future use as a biomarker in conjunction with other measures. Generally, disruption of ion balances in blood is only indicative of acute exposure and does not reflect long term exposure.

During periods of stress, lipids and glycogens may be mobilized as a secondary response to a stress and can be used as an alternative energy source. Determination of these parameters has been used extensively as an indicator of physiological energy status, which can sometimes be translated into responses at the individual level. Several other biochemical indices are available as measures of growth; reproduction; immunity; respiration or genotoxicity. However, virtually none of these measures show high specificity to metals and subsequent relationships to higher level responses are not established.

At the fish population level, chronic exposure to a stress may favour individuals with greater resistance. If there is a genetic component underlying variation in resistance then populations from contaminated and reference areas should differ in the frequencies of certain genotypes. Analysis of allozyme genotypes by electrophoresis has been investigated as a means of detecting disturbances to fish populations due to environmental pollution. However, much further validation of these techniques is required.

In summary, the lack of specificity to metals and low demonstrated ecological relevance make biochemical indices poor candidates for inclusion in ecological studies. **They are not recommended by AETE as tools in a routine mine monitoring program.**

6.5.2 Histopathology

Histopathology is the detailed examination of microscopic alterations in organs and tissues. It is used to examine the association between lesions, their causes and sickness or death in the organism. GlobalTox International Consultants Inc. (1997; Report #2.2.2) carried out a technical evaluation of the use of histopathology in fisheries studies which focused mainly on peer-reviewed literature up to 1996. The tool was also touched upon by EVS (1998). This tool is summarized in Toolbox Summary #12.2 and was examined in the 1995 field program. The Technical Committee decided not to proceed with further field testing of histopathology due to costs and other technical priorities.

The collection of tissues from fish in the field does require special care and consideration. Tissues must be collected fresh and preserved immediately for submission to the analytical laboratory. Although there are a number of highly trained pathologists in government and university laboratories, there are very few in the private sector in Canada that are familiar with fish tissues.

Undertaking histological analysis of fish tissues can provide valuable insight into the reasons for a fish's condition, or even cause of death. Some morphological changes (e.g. hypertrophy of cells) may reflect adaptation to long term exposure to a toxicant. However, pathological changes in fish tissue are generally manifest at more acute exposures and immediately precede death. Lesions induced by metals seem to be most prevalent in gill tissue compared with other organs. However, there are few histological effects that are shown to have high specificity for metals.

Samples of gill, liver and kidney tissues for histopathological examination were collected during the 1995 field program (BEAK 1996). All samples were submitted to the Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, for analysis. Examination of liver tissue showed enlarged hepatocyte nuclear diameter and greater cell area in fish from one Exposure area. These enlargements could reflect an adaptive process by greater synthesis of protein. Northern pike from the Bousquet exposure area had considerably less glycogen possibly suggesting inferior feeding.

No overt lesions were observed in the liver of any fish sampled. The nuclear diameters in livers of yellow perch from one Exposure area were enlarged relative to Reference perch. Cell size was greater in perch from the Reference area due to higher glycogen content, possibly reflecting better feeding conditions than at the Exposure area. These may be considered as general responses to any one of a variety of stressors in the area and definitive conclusions on the effectiveness of histopathology as a monitoring tool could not be made.

Due to a lack of consistent response in the 1995 field survey and the AETE decision to not proceed with additional field studies, **AETE is unable to make recommendations on the use of histopathology of fish tissues in a routine mine monitoring program.**

6.5.3 Fisheries Survey

The Adult Fish Survey (AFS) was reviewed for AETE by EVS Consultants (1998) which provides an excellent guide to the literature available on this subject (AETE Report #2.2.3). The AFS is recognized as a Toolbox containing several tools (Toolbox Summaries #12.1 to 12.6). A fisheries survey component was included as part of each of the three AETE field programs. The AFS has also received considerable attention in Canada in recent years as a controversial component of the Environmental Effects Monitoring (EEM) program for the Pulp and Paper sector under the Federal *Fisheries Act*. The AETE program is able to draw upon the large amount of experience gained by those involved in pulp and paper EEM studies across Canada.

It should be noted that the original Adult Fish Survey (AFS), as described for the Cycle 1 EEM studies, has been changed to simply a Fish Survey (FS) for subsequent cycles. This distinction recognizes that valuable information on growth rates and age to maturity can be provided by juvenile and immature fish. The AETE program uses the broader FS terminology.

This section is divided into the following sub-sections:

- Biological considerations
- General lessons from Pulp and Paper EEM
- Fish growth
- Organ size and fecundity
- Abundance indicators
- Community indicators

i) Biological Considerations

The FS concentrates on measures made at the whole-organism level. Growth, reproduction and condition are the main parameters investigated as indicators of well being that may also be used in population level assessments (Shuter 1990).

Growth is the change in size (weight or length) with time and is usually expressed as an incremental rate process. Growth rates are not constant over time or the lifespan of the fish. The relationship between weight or length with age also differs over the lifespan of a fish. There are two type of growth rates in fisheries:

- true (individual) growth
- apparent (population) growth

The true growth of individual fish can be calculated by measuring the same individual on several different occasions or by back-calculating size at age from scales or other structures. Population growth can be estimated by calculating sizes at various ages. Actual field measurements required include body weight, length (total or fork) and collection of an appropriate structure for age analysis.

If comparing growth (e.g. weight vs. age relationship) between two or more populations the actual comparison is size-at-age rather than comparison of growth rates. It is necessary to recognize this subtle but biologically important difference. Growth rates can differ between males and females and should be examined if present.

Reproductive effects may be determined by measuring gonad weight relative to body size gonadal-somatic index (GSI) or fecundity of females. Fecundity must be standardized for fish size and can be expressed as the # eggs/kg adult female or some similar variable. It is generally measured by counting the number of ova in a subsample of the ovary. Determination of fecundity requires careful measurements either in the field or back in the laboratory. Measuring reproductive effort may be difficult in fractional or batch spawners or in species that do not reproduce each year. A knowledge of the species reproductive biology is important to determine appropriate sampling season if looking at fecundity.

Condition is defined as the weight relative to length of a fish and is an indicator of short term energy storage. It is generally expressed as the *(k)* index, where *k* is generally between 1-2 for most fish, although k <1.0 may be normal for some species. Fish condition can be affected by many factors other than contaminants. It should not be used as a stand alone tool, however, the measures used to calculate condition (weight, length) are readily collected in fish surveys and condition will provide one general indicator of energy storage and fish health.

ii) General Lessons from Pulp and Paper EEM

The objective of the Pulp and Paper EEM program is to assess the adequacy of the national regulations for protecting fish, fish habitat or utilization of the fisheries resources (e.g. tainting, consumption limits). Adequacy was assessed on the basis of magnitude and spatial effects, if any, in receiving environments related to the mill. The EEM regulations required that the program was cyclic in nature with environmental assessments being undertaken once every three years.

The first cycle studies for the Pulp and Paper EEM regulations were completed in 1996. Subsequently, government and industry formed Expert Working Groups (EWG) to review the findings of the first Cycle results and make recommendations for Cycle 2. The Fish Survey EWG reviewed the results of 115 EEM reports (Munkittrick *et al.* 1997) and the interested reader is directed to their report for a useful review and insight into the program findings.

The original AFS in Cycle 1 involved sampling adult fish from one exposure area and a minimum of one reference area. Fish were examined for gross indicators of growth, reproduction and age distribution. It was assumed for the first cycle that 20 males and 20 females would provide sufficient information to provide sufficient data for a statistically defensible design for Cycle 2. Further, it was determined that this information should be gathered for two species.

During Cycle 1 several concerns regarding the FS were identified including:

- fish mobility and migration which might compromise exposure to effluent
- inadequate understanding of natural variability of variables being measured
- difficulty in selecting appropriate sentinel species
- difficulty in obtaining adequate numbers of sentinel species of both sexes
- difficulty in identifying suitable reference area(s)
- confounding influences of multiple discharges or sources near a mill
- lack of understanding of what a meaningful "effect size" might be

Subsequent to development of the EEM regulation, it was decided that the primary purpose of the first Cycle AFS was to obtain estimates of fish variability to allow proper statistical design of subsequent cycles. Secondary objectives were to a) determine the suitability and capture success of the sentinel species, b) evaluate gear suitability, and c) evaluate reference sites. The change in philosophy was significant at the time as it meant that interpretation of the first Cycle results as they pertained to potential effects of individual mills was not required. The original AFS approach suggested that 3 to 5 studies (10-15 year time frame) would be required to determine temporal and/or spatial trends in the data (Hodson *et al.* 1996).

During Cycle 1 only 9% (10/115 studies) were successful in capturing sufficient numbers of both sentinel species. A number of the problems related to catching success are thought to be avoidable by changing sampling time or gear, or choosing an alternative sentinel species.

Selection of an appropriate or suitable reference area was a concern during the Cycle 1 studies. Of 83 freshwater studies, 73 pulp mills discharged to rivers, while 10 discharged to lake environments. Choice of a suitable reference area is likely to be an issue in many mining assessment studies. Many mines in northern Canada discharge directly into relatively small headwater streams. Therefore, obtaining a suitable "upstream" reference habitat will not be possible in a number of instances. Differences in habitat between areas will have a substantial influence on biological variables being measured in both the benthic and fisheries communities. A few pulp and paper Cycle 1 fish surveys used more than one reference area. These studies noted parameter variability between reference areas, as well as between exposure and reference areas. Sampling from multiple reference areas, or sharing references areas between mills is being encouraged for subsequent pulp and paper EEM studies and should be considered for mining assessments.

After Cycle 1, considerable effort was devoted to examining the issue of parameter variability in the FS and determining what constituted an ecological difference between populations. The FS Expert Working Group (EWG) suggested that gonad size and potential effects on reproduction are probably the most important variables to examine. There was a wide range in gonad size responses as a result of exposure to mill effluent. Many studies reported no differences between Exposure and Reference areas. Where there were statistical differences, female gonad weights in Exposed fish ranged from -47.25% to + 187.7 % of the values reported in Reference fish (Munkittrick *et al.* 1997).

The FS Expert Working Group recommended that a ± 25% difference in gonad weight relative to body weight or length between Reference and Exposed areas be adapted as a target effect size. The 25% difference should be regarded as approximate and a range (20-30%) may be more appropriate. The lower limit for the target size is based on two considerations:

i) many fish surveys will not have sufficient statistical power to detect smaller effects, and

ii) differences in gonad weight < 20% may occur naturally between areas. A smaller target size would increase the risk of detecting a false positive response.

Target sizes were not established for other parameters. However, the FSEWG suggested that target effect sizes should be established for growth rather than reproduction in cases where juvenile fish are surveyed. The FS remains a core component of the Pulp and Paper EEM program.

iii) Fish Growth

The 1995 and 1996 field surveys were primarily reconnaissance in nature so detailed evaluations of specific variables were generally not possible. During the 1995 survey at Val D'or, only female white suckers were obtained in sufficient numbers for statistical analysis (BEAK 1996). Fish from the Rivière Bousquet Exposure area had greater body weight at age than fish from the Reference area. No fish were recovered from the exposure area of the Rivière Noire, and it was suggested that the absence of fish might be attributed to ammonia toxicity. Small fish were collected but there were differences in species observed between the four collection areas.

Sufficient fish for statistical analysis were captured at 3 of the 7 sites in 1996 (Dome, Gaspé and Heath Steele). At the Dome site, pearl dace from the Reference area were longer than fish from the Exposure area, but there was no difference in weight. There was no difference ($p > 0.05$) in size of northern redbelly dace between Reference and Exposure areas. The CPUE of all small fish from the Reference area was approximately two times the CPUE at the Exposure area (ESP 1996a).

Juvenile Atlantic salmon were longer and heavier at Reference areas compared with the Exposure area at both the Gaspé and Heath Steele sites (JWEL 1996a; 1996b). Similarly, lake chub collected from the Reference area at Heath Steele were longer, but not heavier, than fish collected from the Exposure area.

In four of six fish species studied during the 1997 survey, fish from the Exposure area were larger at age than fish from the Reference area suggesting faster growth rates. At the Dome Mine, faster growth rate in yellow perch (Figure 6.4) at the Exposure site was coincident with reduced gonad weight suggesting a difference in the proportional allocation of energy between somatic growth and reproduction in exposed fish.

Fish growth rate was considered only partially effective at demonstrating a mine-related effect at the three sites tested in 1997 (Table 6.5). This was largely due to the fact that the direction of change did not appear consistent with an expected mine-related effect. It should be emphasized that not detecting a biological response should not always be considered a failure of the tool. Rather, it may be the appropriate measure that there in fact was no relevant whole organism consequence of the exposure. This applies to growth rate as well as organ size and fecundity (next section).

It is apparent that fish growth rate is a fundamental parameter of fish biology, and therefore, **AETE recommends that growth rate and size at age are suitable tools in routine mine monitoring studies.**

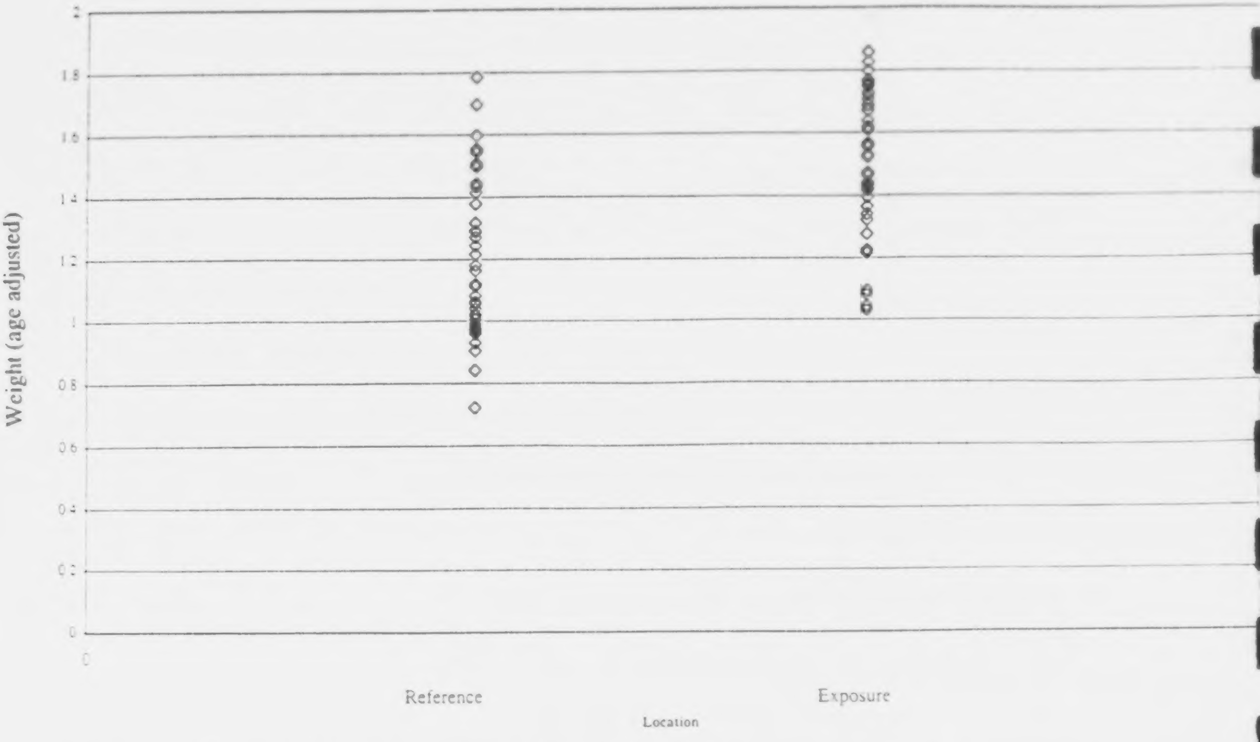


Figure 6.4 Comparison of age-adjusted weight of yellow perch from Exposure and Reference areas, Dome Mine, 1997.

iv) Fish Organ Size and Fecundity

Different organs have been examined in fish studies including spleen, gill and skeleton, but most focus has been on the liver and gonads. This tool measures the size of the whole organ relative to the total size of the fish and, therefore, differs from histolopathogy which examines microscopic alterations in tissues or organs. These measurements are also considered part of a fish survey but are discussed here as specific measurement endpoints or tools. Fish organ examination was reviewed for AETE by EVS Consultants (1998; Report #2.2.3) and is summarized in Toolbox Summary #12.3. These parameters were also measured in the 1995 and 1997 field programs.

The relative size of fish liver and gonad has been used to indicate energy storage and reproduction, respectively. Liver enlargements may occur as a result of high carbohydrate diet or increased enzyme activity for detoxification of a compound (Dixon *et al.* 1987). Increased relative liver size can be detected by calculating the Hepatosomatic Index (HSI) which expresses liver weight as a proportion (%) of the total fish body weight. Liver weight is easily measured in the field for large fish specimens that have distinct livers, but may be more difficult for small specimens or species that have diffuse livers attached throughout the intestine (e.g. white suckers).

Atrophy or decreased gonad size can result from food limitation or reproductive dysfunction. In other instances, gonad size, especially in females, may be increased as a physiological response to stress. In either case, gonad size between sample populations can be expressed as the Gonadosomatic Index (GSI) which measures gonad weight as a proportion of total fish body weight. Ideally, the GSI would be measured in fish just prior to spawning.

There is increasing interest in using small fish species as sentinel species in fish surveys. Attention to precision and sensitive equipment is required for measuring the weights of individual organs from small fish. In addition, some minnows and other small fish species are fractional spawners that lay eggs more than once during the year. The study designer should be aware of this situation and attempt to collect gonad information before the first spawning event occurs.

The ecological relevance of the LSI or GSI may be debated, however, it should be apparent that factors affecting the reproductive performance of individual fish also have the potential to affect populations. Further discussions of the GSI related to the Pulp and Paper EEM program and effect sizes considered to be ecologically significant were presented above.

During the 1995 field survey it was observed that female white suckers from the Exposure area had greater gonad weight at length and greater egg size than fish from the Reference area (BEAK 1996).

Changes in liver size could possibly show an increase, decrease or no change in response to exposure to mine effluent. An increase in size could potentially occur in response to increased hepatic detoxification activity, disruption of lipid and glycogen metabolism or simply increased energy storage. A decrease in liver size could be interpreted as energy storage depletion. These changes might occur as a result of a direct effect from exposure to metals or an indirect effect due to differences in food availability.

During the 1997 surveys, increased liver weight was observed in pearl dace and yellow perch in the exposure area at the Dome site, however, there was no statistical difference when the liver weight was adjusted for body weight in either species.

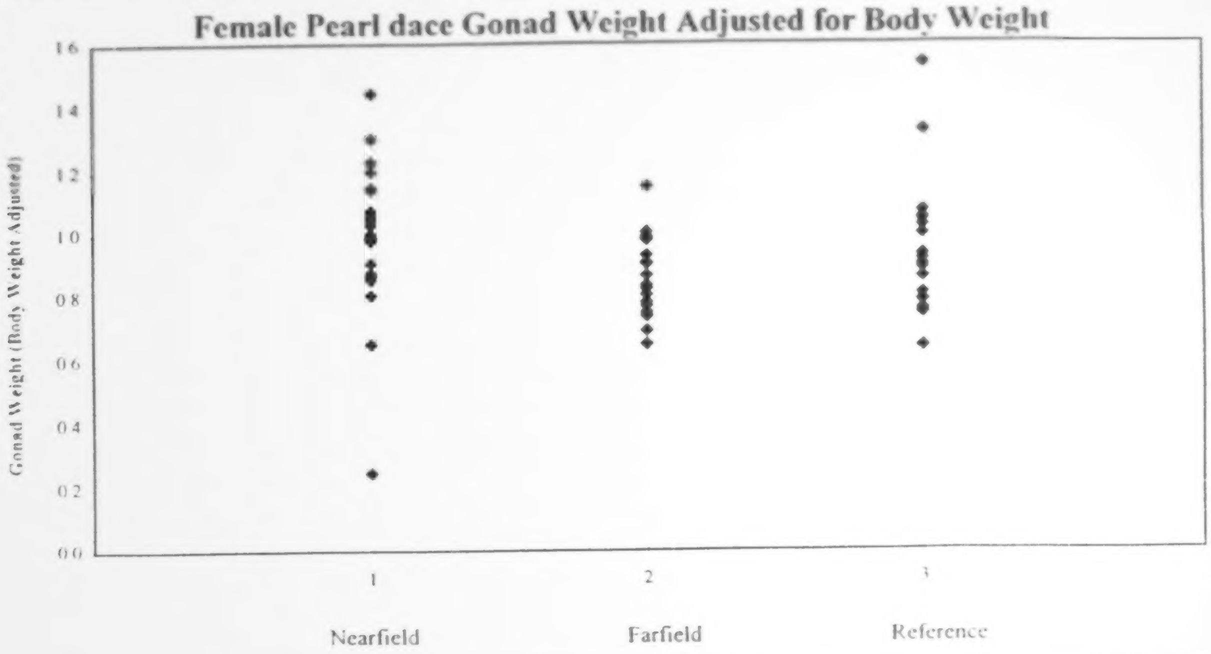
At Mattabi, liver weights at age and size were greater in male white suckers from the exposure area. In contrast, liver weight was lower in both age and size adjusted northern pike from the exposure area. Therefore, the tool was ranked as partially effective (Table 6.5) because although differences occurred, they were found only in one sex of each species, and the species effects were in different directions (BEAK/Golder 1998c). The species difference may be related to differences in habitat preference and feeding relationship although examination of supporting data (e.g. benthic density, fish abundance) provided no obvious explanation.

Table 6.5. Summary of Effectiveness Rankings for Fish Health Indicators

	Fish Health Indicators				
Mine Site	**Fish Growth**	**Liver Weight**	**Gonad Weight**	**Fecundity**	**Comments**
Dome Mine	P	P	P	P	Significant increase in length and weight of perch at age in exposure area. Significant increase in length and weight in exposure area for pearl dace. In yellow perch, no significant reference-exposure difference in gonad weight (males and females) and fecundity at age. Livers significantly larger in exposure yellow perch. Gonad weight (body weight adjusted) for males and females lower in exposure area. Liver weight adjusted for body weight showed no change. Significantly larger pearl dace gonad and liver weights in exposed females and males. Pearl dace fecundity higher in exposure area. Female dace body weight-adjusted gonad weight and fecundity lower in exposure area. Liver weight unchanged when adjusted for body weight.
Heath Steele	X	NT	NT	NT	Young-of-the-Year (YOY) salmon were smaller in high density reaches (below barriers). Effect persists at later ages. No impairment of growth in salmon or blacknose dace.
Mattabi Mine	X	P	X	X	No significant differences in growth of white sucker. Significantly larger pike in exposure area. White sucker liver significantly larger in exposure fish. Gonad weight (body-weight adjusted) slightly smaller in exposed male sucker. Liver and gonad weight and fecundity in pike all significantly higher in exposed fish. Effects of exposure in pike not consistent with adverse impact.
Myra Falls	NT	NT	NT	NT	

NT - Not tested
√ - Effect demonstrated
X - Effect not demonstrated
P - Effect partially demonstrated
* - More effective at demonstrating an effect

At Dome Mine, gonad weight and fecundity (both adjusted for body weight) of pearl dace were lowest in the near-field area compared with far-field and reference fish (Figure 6.5), but no differences were observed for yellow perch. At Mattabi Mine, there was no significant difference in gonad weight or fecundity for northern pike or white sucker.



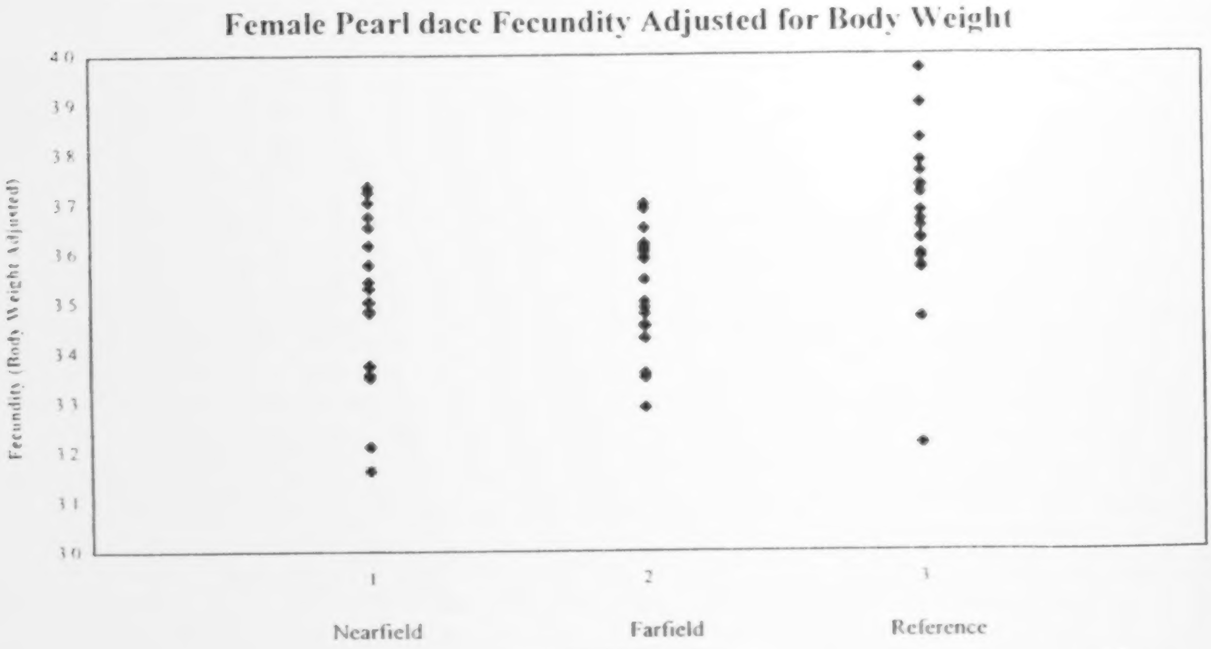


Figure 6.5. Gonad weight (upper graph) and fecundity (lower graph) in Pearl dace at the Dome Mine, 1997.

Responses of organ size and fecundity were not clearly evident at all sites which may be the appropriate measure if conditions were not sufficient to induce a response. Factors other than exposure to mine effluent (e.g. habitat differences, food availability, competition) can also influence results.

These parameters are fundamental measures of general fish health and, therefore, **AETE recommends that Gonadosomatic Index, Liver Somatic Index and fecundity are suitable tools for a routine mine monitoring program.**

v) Abundance Indicators

The FS described above focused on the whole organism, while other approaches are available to examine fish population or community trends. Traditional techniques to estimate the absolute numbers of fish within a population include mark-recapture studies which require extensive time and effort, and do not lend themselves to routine assessments. In place of these, the relative abundance of fish species can be documented by abundance indices with Catch-Per-Unit-Effort (CPUE) being one of the most common. The CPUE index was reviewed for the AETE program by EVS Consultants (1998; Report #2.2.3) and is summarized in Toolbox Summary #12.5. The relative abundance of fish from Exposure and Reference areas was documented in the 1996 and 1997 field programs.

Estimates of fish abundance are highly ecologically relevant. The major limitation of abundance estimates is that variability can be very high and large sample sizes are required for reliable estimates. When sampling occurs over a relatively short period, the catch (and resultant CPUE estimate) is subject to diurnal and seasonal fish movement patterns. Sampling gear also has an obvious profound effect on CPUE estimates. Abundance is also a non-specific response and will be influenced by many other factors (e.g. indirect habitat alterations) in addition to effluent exposure. All these factors must be taken into account when designing a fish survey where CPUE data will be collected, and when the CPUE results are being interpreted.

Fish abundance indicators were measured at two of the mine sites (Heath Steele, Mattabi) during the 1997 surveys. These included Catch-per-unit-effort (CPUE) and Biomass-per-unit-effort (BPUE). Both indicators were effective at demonstrating exposure to mine effluent at Heath Steele especially when all fish species were considered together (Table 6.6). At Mattabi, both CPUE and BPUE showed no statistical difference between reference and exposure areas. That may not be unexpected, however, since the tissue metal level data indicated little exposure to metals at this site which is consistent with the low metal levels measured in water. Similarly, there was no observed effect on fish health indicators (see above). Therefore, it is possible that these tools at Mattabi are effective in demonstrating that the mine is not impacting fish resources in the area.

It appears that potentially valuable data on relative abundance can be collected incidental to the fish survey. **Therefore, recording CPUE of fish species is recommended to provide further information that may be used in a weight-of-evidence approach to evaluate the impacts of mining effluents on aquatic systems.**

Table 6.6 Summary of Effectiveness Rankings for Catch/Biomass-Per-Unit-Effort

Mine Site	CPUE[1]		BPUE[2]		Comments
	Individual Species	All Fish	Individual Species	All Fish	
Dome Mine	NT	NT	NT	NT	Qualitative analysis. Not effective but due to habitat differences and introduced species.
Heath Steele	P	√	P	√	Fish CPUE (all taxa combined) was reduced with degree of exposure and exposure area means were lower than reference means.
Mattabi Mine	X	X	X	X	No significant effect of mine exposure on fish abundance.
Myra Falls	NT	NT	NT	NT	

[1] Catch-per-unit-effort (numbers).
[2] Biomass-per-unit-effort.
NT - Not tested.
√ - Effect demonstrated.
X - Effect not demonstrated.
P - Effect partially demonstrated.
* - More effective at demonstrating an effect.

vi) Community Indicators

Fish populations do not necessarily decline when exposed to chronic contamination. For example, predatory species (trout, walleye) are sometimes more sensitive to metal toxicity than forage species (minnows, white suckers). If the higher trophic levels are removed or reduced, the relative abundance of the lower trophic levels will increase substantially. In this situation, there has been a shift in community structure as shown by relative abundance of all the fish species.

Various methods to examine fish community composition have been proposed and were reviewed under the auspices of Fish Community Surveys (FCS) by EVS (1998). The FCS approach includes the Index of Biotic Integrity (IBI) as proposed by Karr *et al.* (1986) and the U.S. EPA protocol for rapid bioassessment. Fausch *et al.* (1990) identified four approaches to FCS:

- indicator taxa/guilds
- simple indices (i.e. richness, diversity)
- multivariate analyses
- the IBI and related summary indices

The use of IBI and other indices is rarely useful for communities with few fish species as is often the case in northern Canadian lakes, where many mines are located. The use of FCS was not formally included as part of the AETE field programs, although EVS (1998) suggested that FCS should be considered in a mine monitoring program on a regional basis. The use of FCS or even CPUE has not been adapted in Canada as part of the Pulp and Paper EEM program.

The AETE does not recommend that fish community indicators be formally included in a routine monitoring program. However, information on general species composition should be maintained as it may be useful for qualitative interpretation of habitat and mine-related effects.

7. ARE THE CONTAMINANTS IN THE SYSTEM CAUSING THE RESPONSE?

This question cannot be answered directly through the application of specific monitoring tools evaluated in this study, or directly through any of the hypotheses tested. Rather, the question can be evaluated only by a weight-of-evidence provided by affirmative responses to the first three questions, and by the strength of the correlations between exposure indicators and biological responses. These are the subject of hypotheses H9 through H13 (Table 7.1) which are discussed in greater detail in this chapter.

Table 7.1 Tool Integration Hypotheses

Integration of tools:	
Relationship between water quality and biological responses:	
H_9:	*The strength of the relationship between biological variables and metal chemistry in water is not influenced by the choice of total vs. dissolved analysis of metals concentrations.*
Relationship between sediment chemistry and biological responses:	
H_{10}:	*The strength of the relationship between biological variables and sediment characteristics is not influenced by the analysis of total metals in sediments vs. either metals associated with iron and manganese oxyhydroxides or with acid volatile sulphides.*
Relationship between sediment toxicity and benthic invertebrates:	
H_{11}:	*The strength of the relationship between sediment toxicity responses and in situ benthic macro-invertebrate community characteristics is not influenced by the use of different sediment toxicity tests, or combinations of toxicity tests.*
Metals or metallothionein vs. Chemistry (receiving water & sediment):	
H_{12}:	*The strength of the relationship between the concentration of metals in the environment (water and sediment chemistry) and metal concentration in fish tissues is not different from the relationship between metal concentration in the environment and metallothionein concentration in fish tissues.*
Chronic Toxicity - Linkage with Fish and Benthos monitoring results:	
H_{13}:	*The suite of sublethal toxicity tests cannot predict environmental effects to resident fish performance indicators or benthic macro-invertebrate community structure.*

7.1 Methods of Hypothesis Testing

H9 through H12 - Tool Integration Hypotheses

Hypotheses H9, H10 and H11 address the relative ability of two monitoring tools to detect a mine effect in the form of a correlation between responses measured and exposure. For example, in H9, dissolved metal in water was compared to total metal in water, for each of the key metals, to determine whether these two monitoring tools differ in their correlation with a response measure, such as number of taxa. Correlation analysis was used to address this hypothesis, as described below.

The squared coefficient of correlation (r^2) between the response measure (Y) and each predictor variable (X1 or X2) indicates the proportion of variance in the response measure that is explained by the predictor. The best predictor, for each pair compared, is the one which explains the highest proportion of variance (i.e. has the highest r^2).

Hypothesis H9 was tested by correlation between benthic or fish values and metal concentrations in water (dissolved or total) from stations in areas sampled (reference, near field, far field). Hypothesis H10 was tested in a similar manner by correlation of benthic or fish index values versus sediment chemistry correlations and sediment toxicity versus sediment chemistry correlations, based on near-field, far-field and reference stream data. The sediment chemistry tools include total metal concentrations (hydrogen peroxide/nitric acid extraction), partial metal concentrations (hydroxylamine hydrochloride extraction) and the ratio of the molar sum of simultaneously extracted metals (SEM) and acid volatile sulphide (AVS). Metals included in the SEM value are Cd, Cu, Ni, Pb and Zn. These are the metals normally contributing to toxicity and potentially rendered non-bioavailable by the formation of metal monosulphides.

Hypothesis H11 examined the remaining component of the "sediment quality triad" - the correlation between benthic indices and sediment toxicity - based on near-field, far-field and reference stream data. The toxicity tests included amphipod (*Hyalella azteca*), chironomid (*Chironomus riparius*) and oligochaete (*Tubifex tubifex*) tests on sediment samples from each stream station.

Hypothesis H12 examined the correlation between water and sediment chemistry measurements and concentrations of metals and metallothionein in fish tissues. For fish, station means were used as values in order to permit pairing with water and sediment chemistry values.

H13 - Chronic Toxicity - Linkage with Benthic and Fish Results

Hypothesis H13 addresses the ability of a particular effluent toxicity testing tool to predict a mine effect that has been otherwise demonstrated (e.g. a benthic index response to exposure). For example, H13 might address whether a specific benthic response can be predicted from effluent toxicity to *Ceriodaphnia*, *Selenastrum*, fathead minnow or duckweed.

In order to test this hypothesis, it is necessary to estimate the receiving water toxicity to each species in the near-field and far-field areas, based on the effluent toxicity information and the expected downstream dilution of effluent close to the time of the survey. This could only be statistically evaluated at Heath Steele. At Myra Falls and Dome Mine this hypothesis was only evaluated qualitatively due to study design limitations.

Water toxicity, like effluent toxicity, can be expressed as a % inhibition (i.e. for *Ceriodaphnia* as % inhibition of reproduction). The % inhibition increases with effluent concentration. The IC25 concentration produces 25% inhibition and the IC50 concentration produces 50% inhibition. These two concentrations, obtained from the effluent toxicity test, define the % inhibition vs. concentration relationship. This relationship was used to estimate the % inhibition that would be expected at each effluent concentration that exists in downstream reaches.

Water toxicity was estimated in this manner for each exposure area downstream of the mine, based on three different effluent samples and up to four different toxicity test methods (*Ceriodaphnia*, fathead minnow, algae, duckweed). Each of these toxicity variables was tested for correlation with each of the field measurements of biological response, such as fish CPUE, and plots were produced to illustrate some of the stronger relationships.

Triad Hypothesis

The "triad" approach also addresses the issue of whether chemicals may be responsible for biological effects in the study area. This hypothesis was not explicitly articulated in the original 13 hypotheses (Table 3.2) but it is implicitly involved in H9 through H13. The basic approach to evaluation of the triad hypothesis is to simultaneously examine three types of correlations:

- chemical - toxicological (C-T)
- toxicological - biological (T-B)
- chemical - biological (C-B)

Statistical approaches to evaluation of the triad approach have traditionally followed Green and Montagna (1993) and Chapman (1996). One approach is to examine the three bivariate correlations (C-T, T-B, C-B) for different sets of data. Then, the overall evaluation is based on "weight-of-evidence" considerations. This approach can be tedious with large datasets. A different holistic approach was applied in this study using principal components analysis (PCA) to reduce the large number of variables to one or two dominant components. Further statistical tests were applied to determine if there was overall concordance across the three arms of the triad (BEAK/Golder 1998c).

It should be noted that various statistical procedures were applied for data analysis throughout the AETE program. However, the program did not consider statistics as monitoring tools *per se*, nor did it evaluate statistics on this basis.

7.2 Result of Hypothesis Testing

Hypothesis H9 - Relation between water quality and biological variables

The concentrations of either total or dissolved metals were compared with biological responses (fish CPUE, fish growth, fish organ size, benthic community descriptors) to test H9.

A correlation of both total and dissolved metals with biological indices was demonstrated at Myra Falls and Heath Steele, while it was partially demonstrated at Dome and Mattabi (Table 7.2). For example, at the Dome Mine, gonad size of female pearl dace (adjusted for body size) was significantly lower in the near-field and far-field fish compared with reference fish. Similarly, weight-adjusted fecundity was lowest in the near-field and highest in the far-field fish which is consistent with a mine-related effect (Figure 6.5).

Table 7.2. Total Versus Dissolved Metals as Predictors of Biological Responses (Hypothesis H9)

Mine Site	Total Metal	Dissolved Metal	Comments
Dome Mine	P	P	Total and dissolved As negatively correlated with fecundity whereas Mg and Ni positively correlated. No mine-related correlations with benthic indices except for negative correlations of total and dissolved Co, Cu, K, Mg, Ni with % chironomids. Body weight-adjusted female gonad weight negatively correlated with Co and Cu. Dissolved and total metals equally effective, although limited. Correlations may be limited due to the fact the mine only discharges sporadically and was not discharging at the time of the survey.
Heath Steele	√	√	Numbers of total benthic taxa and EPT taxa reduced and dominance of tolerant chironomids increased with increasing metal in water. Fish CPUE, BPUE and number of fish taxa decrease with increasing metal in water. Total and dissolved metals (Zn, Cd, Cu, Pb, Al) correlated with biological effects. Strength of the relationships similar for dissolved and total metals versus biological responses.
Mattabi Mine	P	P	Similar negative correlations for a limited number of dissolved and total metals (Cu, Fe, Mg, Zn). Pb concentrations of both appeared unrelated to most biological effects. H9 to be interpreted with caution due to study design limitations (CI design) for number of fish taxa.
Myra Falls	√	√	Only one exposure level; therefore, correlations not possible. Dissolved and total metals higher in exposure area where effects on benthos were observed. Dissolved metals were a high percentage of total metal; therefore, correlations with benthic effects would have been similar. H9 assessed qualitatively.

√ - Effect demonstrated

P - Effect partially demonstrated

At Heath Steele there were a number of total and dissolved metals that were mine-related and correlated with biological effects. The strength of correlations which appeared to be mine-related were similar for both total or dissolved metal concentrations. For example, at Heath Steele the correlation coefficient of CPUE for all fish species was - 0.89 with both total or dissolved Cu. The r value for total and dissolved Cu with the benthic EPT index was -0.754 and -0.746, respectively. The correlations were similar because virtually all metals were present in the dissolved form at these sites. Therefore, it was not possible to evaluate relationships where the concentration of dissolved metals was lower than total metals.

Since the strength of the correlation with biological responses was similar for both total and dissolved metals at these sites, and total metals were much easier to collect and analyze (see Section 4.4), the results of testing H9 support the recommendation to use total metal levels in receiving water versus dissolved metal concentrations. However, because dissolved and total metal concentrations tended to co-vary and dissolved metals represented a high percentage of total metals at the sites tested, the relative effectiveness of total versus dissolved metals requires further testing.

Hypothesis 10: Relationship between sediment chemistry and biological responses

This hypothesis investigates the linkage between sediment chemistry and biological responses. For the purpose of this hypothesis, sediment toxicity is also considered a biological response and is discussed first under this heading.

Sediment toxicity was generally correlated with a number of sediment metals (Table 7.3), with a similar correlation for total and partial metal concentrations. Since total metals are easier and less expensive to measure, and directly comparable with agency sediment quality guidelines, the results of the hypothesis testing support the measure of total metals in sediments versus partial metal concentrations.

Table 7.3. Comparison of the Relationships Between Sediment Chemistry and Sediment Toxicity Results (Hypothesis 10)

Mine Site	Sediment Toxicity Tools			Comments
	Hyalella azteca	*Chironomus riparius*	*Tubifex tubifex*	
Dome Mine	√	NT	NT	*Hyalella* mortality positively correlated with total and partial As, Co, Cr, Cu, Fe, Hg, Mg and Ni. *Chironomus* and *Tubifex* not tested because they showed no mine response.
Heath Steele	NT	NT	NT	
Mattabi Mine	NT	√	√	Similar correlations for total and partial metals with sediment toxicity results (*Chironomus* and *Tubifex* sublethal endpoints). Total metals slightly better correlated than partial metals. *Hyalella azteca* not tested because it showed no mine-related or area effects.
Myra Falls	√	√	√	Sediment toxicity was correlated with both total and partial metals for As, Cd, Cu, Zn. Total metals were better correlated with toxicity than partial metals overall.

NT - Not tested.
√ - Effect demonstrated.

Total and partial metal concentrations also proved equally effective in demonstrating a linkage between sediment chemistry and benthic indicators (Table 7.4). The SEM/AVS ratio was not a good predictor of either toxicity or biological responses.

At all three sites, significant sediment toxicity (mortality) occurred in some sediment tests at SEM/AVS ratios below 1 which is contrary to the SEM/AVS model. The SEM/AVS ratio was developed to predict acute sediment toxicity but not necessarily chronic effects including effects on the benthic community. In general, SEM/AVS ratios < 1 may reflect not-toxic sediment conditions because some of the key metals (e.g. Ni, Pb, Cu, Zn, Cd) which are often associated with sediment toxicity will be in the sulphide form which reduces their bioavailability. However, it is possible that sediments with SEM/AVS ratios < 1 will exhibit toxicity due to the presence of other metals (e.g. As, Hg) which are not included in the SEM analysis.

Table 7.4. Relationship Between Sediment Chemistry Tools and Biological Responses (Hypothesis H10)

Mine Site	Sediment Chemistry Tool			Comments
	Total Metal	Partial Metal	SEM/ AVS	
Dome Mine	P	P	X	Total and partial metals similarly correlated with benthic indices and sediment toxicity. Only a few metals correlated with benthic effects. *Hyalella* mortality positively correlated with total and partial As, Co, Cr, Cu, Fe, Hg, Mg and Ni. No correlation with SEM/AVS and benthos or toxicity results. SEM/AVS ratio was not a good predictor of acute sediment toxicity.
Heath Steele	√	NT	NT	Numbers of benthic taxa and EPT taxa reduced and dominance of tolerant chironomids increases with increasing metals in periphyton. Fish CPUE, BPUE and number of taxa decrease with increasing metals in periphyton. For most fish and benthic indices, relationships were stronger with metals in water than metals in periphyton
Mattabi Mine	√	√	X	Correlations significant for total and partial metals (As, Cd, Cu, Fe, Pb, Ni, Se, Zn) with benthic community effects and sediment toxicity results (*Chironomus* and *Tubifex* sublethal endpoints). Total metals slightly stronger correlated than partial metals. No correlation of SEM/AVS ratios with benthos or toxicity results.
Myra Falls	√	√	X	Benthic indicators and sediment toxicity were correlated with both total and partial metals for As, Cd, Cu, Zn. Correlation coefficients for total and partial metals were similar for benthic indicators. Total metals were better correlated with toxicity than partial metals. The SEM/AVS ratio did not correlate with either benthic indicators or with sediment toxicity and was not an effective predictor of acute toxicity

NT - Not tested.
√ - Effect demonstrated.
X - Effect not demonstrated.
P - Effect partially demonstrated.

SEM/AVS ratios > 1 often reflect toxic sediments because there is insufficient sulphide to react with the bioavailable metals to make them less toxic. During this program, at all sites where the SEM/AVS ratio was measured, it was unreliable as a predictor of sediment toxicity (Table 7.4). The results of the hypothesis testing support the recommendation to measure total metal concentrations in sediments, and not partial metals or the SEM/AVS ratio.

Hypothesis 11: Relationship between sediment toxicity and benthic invertebrates

This hypothesis investigated the linkage between sediment toxicity test results and benthic responses measured in the receiving environment.

The *Hyalella* test results were effective for predicting impacts to the benthic community at Dome Mine and Myra Falls but not at Mattabi (Table 7.5). However, other biological indices at Mattabi also indicated there was little biological impact, therefore, despite elevated metal levels at this site the bioavailability may be low.

Sediment toxicity was correlated to benthic biological measures in 4 of 9 cases and there was variability among sites as to which tests were most effective (Table 7.5). This would support the concept of applying a suite of sediment toxicity tests for detailed impact assessment.

Table 7.5. Comparison of the Relationships Between Sediment Toxicity Tools and Benthic Invertebrate Communities (Hypothesis H11)

Mine Site	Sediment Toxicity Tools			Comments
	Hyalella azteca	*Chironomus riparius*	*Tubifex tubifex*	
Dome Mine	√	X	X	*Hyalella* mortality and growth correlated with most benthic indices. *Hyalella* test effective in predicting impacts on benthic community.
Heath Steele	NT	NT	NT	
Mattabi Mine	X	X	√	*Tubifex* reproduction showed strongest correlations with benthic metrics supporting cause-effect linkages *Chironomus* growth showed some linkage with benthos but the direction of the correlation was inconsistent with impact.
Myra Falls	√	X	√	Benthic indicators (harpacticoids and *Pisidium*) were correlated with toxicity test results for *Tubifex* reproduction (positive correlation) and for *Hyalella* mortality (negative correlation). Chironomid mortality was not correlated with benthic indicators.

NT - Not tested.
√ - Effect demonstrated.
X - Effect not demonstrated.

Hypothesis 12: Metals or metallothionein versus water or sediment chemistry

This hypothesis explores the linkage between metal or metallothionein levels in fish tissue and metals in water or sediments. A summary of findings is provided in Tables 7.6 and 7.7, respectively.

There were some correlations between either total or dissolved metal levels in water and fish tissue metal levels at three sites. (Note this hypothesis was not tested at Myra Falls). There were some correlations between total or dissolved metals in water and fish MT levels at Heath Steele and Mattabi, but not at the Dome Mine (Table 7.6). The generally greater proportion of significant correlations of metals in water and tissue metals versus tissue MT supports the recommendation to monitor the former rather than the latter.

At all three sites, correlations between the variables were similar for both total and dissolved metal concentrations. Therefore, the results of this hypothesis test demonstrate there is no advantage in measuring dissolved metal levels over total metal concentrations based on the AETE field program. However, conditions at the sites were not conducive to rigorously test the relative effectiveness of total versus dissolved metal concentrations in water.

Table 7.6. Total Versus Dissolved Metals in Water as Predictors of Metallothionein and Metal Bioaccumulation (Hypothesis H12)

Mine Site	Total Metal	Dissolved Metal	Comments
Dome Mine	√	√	Total and dissolved Co, Cu, Ni correlated with pearl dace viscera. No mine-related response between MT and aqueous metals. No overall difference in strength of the correlations of total and dissolved aqueous metals with viscera concentrations.
Heath Steele	√	√	MT in wild and caged salmon viscera correlated with metals in water; metals in salmon did not correlate with metals in water. MT in blacknose dace viscera correlated with Pb in water. Strength of the correlations of total and dissolved metals and MT in tissues similar.
Mattabi Mine	√	√	In sucker, only Pb and Zn in gill were correlated with aqueous metal concentrations. MT concentrations in sucker tissues were unrelated to exposure concentrations. In pike, Pb in kidney and gill, and Zn in muscle, were correlated with aqueous concentrations of the same metals. Pike MT levels in kidney and gill were correlated with Cd, Pb and Zn in water. These metals in tissue showed similar correlations with corresponding metals in water.
Myra Falls	NT	NT	

NT - Not tested.

√ - Effect demonstrated.

The concentration of total and partial metals in sediments was related to tissue metal levels at Dome, but for only two of the many metals examined. There was no relationship to fish MT levels at Dome (Table 7.7). At Heath Steele, the levels of Zn, Cu and Pb in periphyton (used as surrogate for sediments) were correlated with levels in viscera of Pearl dace, but no relation existed with metal levels in salmon. Therefore, the relation is considered only partially effective (Table 7.7).

Based on these results, there were only limited relationships between sediment metals and fish tissue metal or MT concentrations. The data do not indicate a preference for either total or partial metals in sediments, therefore, there is no change to the recommendation made in Section 4.5 to measure only total metal levels in sediments for routine monitoring.

Table 7.7. Relationship Between Sediment Chemistry Tools and Tissue Metal or Metallothionein (Hypothesis H12)

Mine Site	Sediment Chemistry Tool			Comments
	Total Metal	Partial Metal	SEM/ AVS	
Hypothesis H12				
Dome Mine	P	P	X	No mine-related response between MT and sediment metals. Sediment total and partial Ni and Co correlated with viscera metals, also partial arsenic. No overall difference in correlations of total and partial sediment metal versus viscera concentrations, although the number of significant mine-related correlations was few.
Heath Steele	P	NT	NT	Metals in salmon did not increase with metals in periphyton. Zn, Cu and Pb in dace viscera correlated with metals in periphyton.
Mattabi Mine	NT	NT	NT	
Myra Falls	NT	NT	NT	

NT - Not tested.
√ - Effect demonstrated.
X - Effect not demonstrated.
P - Effect partially demonstrated.

Hypothesis 13: Relationship of chronic toxicity results to fish or benthos responses

This hypothesis examined the link between effluent sublethal toxicity test results and in-stream biological (fish or benthos) responses. However, it could only be statistically tested at one site (Heath Steele) and qualitatively tested at Dome and Myra Falls due to the study designs.

In general, all toxicity tests were correlated with a biological response at a mine site (Table 7.8). Fathead minnows proved the least effective at "predicting" in-stream effects, which is consistent with the fact that they were demonstrated to be the least sensitive species to mine effluent toxicity (Section 6.1). However, some observations are noteworthy:

- toxicity in fathead minnows was observed at concentrations in the receiving environment where fish CPUE and BPUE were affected,
- thresholds for growth impairment in fathead minnows in Dome effluent occurred at concentrations greater than those found downstream under conditions of effluent discharge. This is consistent with the observation of no impairment of growth in yellow perch or pearl dace downstream of Dome, and
- benthic community effects occurred at Dome, Myra Falls and Heath Steele at effluent exposure concentrations consistent with sublethal and/or lethal effects in the *Ceriodaphnia* tests.

Figure 7.1 illustrates an example of the relationships measured between sublethal toxicity test results and fish CPUE at Heath Steele. At all sites, the plant tests were also consistent with effects observed in biological communities in the receiving environment.

Table 7.8. Effectiveness of Sublethal Toxicity Tests in Corresponding with *In Situ* Biological Effects (Hypothesis H13)

Mine Site	Effluent Toxicity Tools				Comments
	Ceriodaphnia	*Selenastrum*	*Lemna*	*Pimephales*	
Dome Mine	√	√	√	P	Effluent toxicity tests appeared effective in predicting effects on benthic communities and in predicting that there would be no effects on fish growth. Fathead minnow test was not effective in predicting body weight-adjusted effects in fish.
Heath Steele	√	√	√	√	Fish CPUE, BPUE and number of taxa decrease with predicted water toxicity to algae, *Ceriodaphnia*, duckweed or fathead minnow. Dominance of pollution-tolerant chironomids increases with predicted water toxicity. Other benthic indices not correlated with predicted toxicity. The four toxicity tests produce similar biology vs predicted toxicity correlations when effluent is sublethally toxic. Fathead minnow test is less sensitive than other tests.
Mattabi Mine	NT	NT	NT	NT	
Myra Falls	√	√	√	X	Benthic effects were observed in the exposure area of Myra Creek, and occurred at aqueous metal concentrations producing chronic toxicity in *Ceriodaphnia*, *Lemna* and *Selenastrum*. Therefore, these tests appeared to effectively predict benthic effects. No fathead minnow response occurred in any test (lethal or sublethal).

NT - Not tested
√ - Effect demonstrated
X - Effect not demonstrated
P - Effect partially demonstrated

The qualitative study results for Dome and Myra Falls, and the approach used for hypothesis testing at Heath Steele, suggest that effluent sublethal toxicity tests may prove to be a useful tool for predicting biological effects in the receiving environment. The statistical methods and study design parameters used to test this hypothesis are in themselves useful for providing further weight-of-evidence that contaminants in the system are responsible for a particular response.

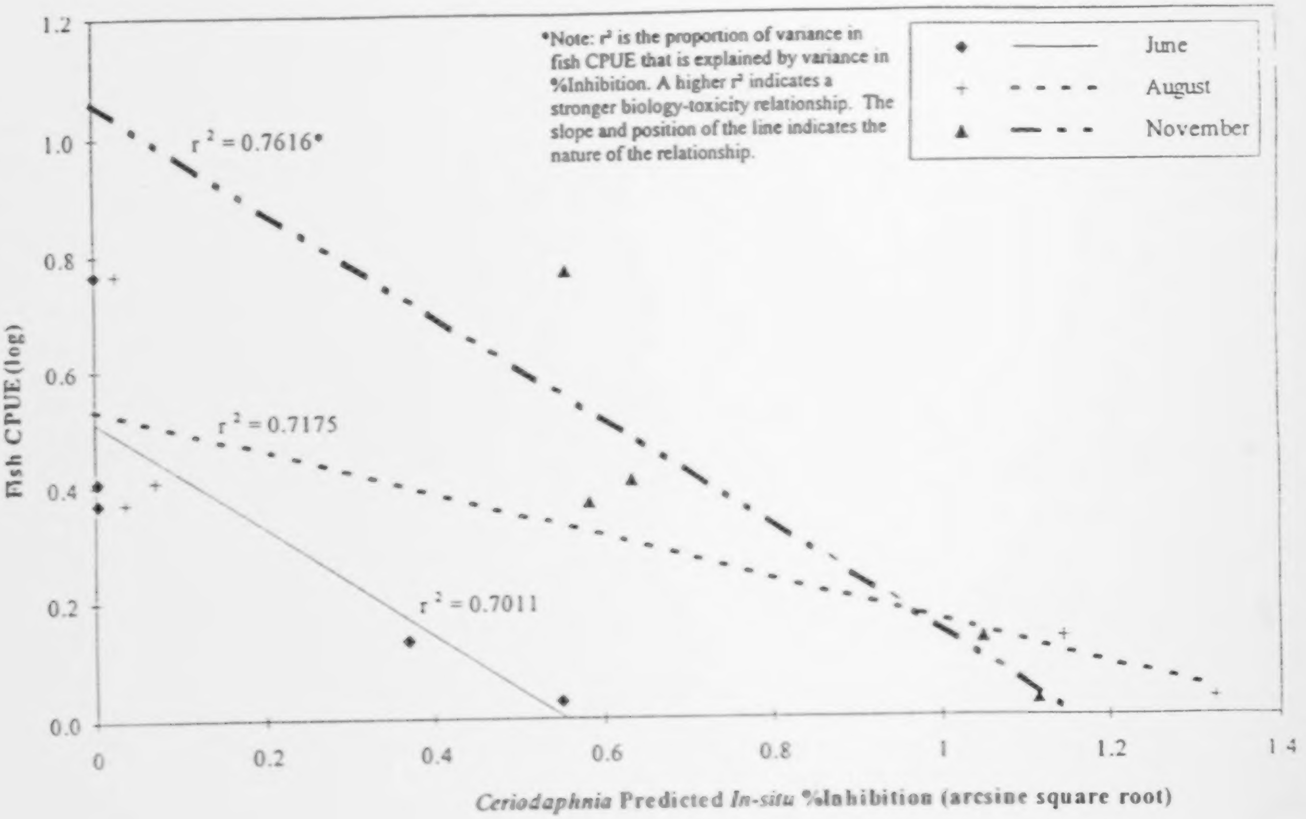


Figure 7.1. Fish CPUE versus *Ceriodaphnia* Inhibition at Heath Steele.

Sediment Quality Triad

At each mine site, the relationships between each "arm" of the sediment quality triad were examined. Figure 7.2 illustrates the results of statistical testing between the three components (C-T, B-T, B-C) for Myra Falls using one approach. In each comparison there was a significant correlation between the two variables. The correlation between sediment toxicity and chemistry was stronger than the other two arms of the triad possibly reflecting different causative agents or an acclimation effect for the benthos.

At the Dome Mine the linkages were strong between sediment chemistry and toxicity, and between toxicity and the benthic community response. However, the linkage between sediment chemistry and benthic community response (C-B) was not as strong (Table 7.9).

At Myra Falls, the linkages were strong between sediment chemistry and both the benthic community response and sediment toxicity. The correlation between sediment toxicity and benthic community response was somewhat weaker, possibly reflecting different causative mechanisms.

Table 7.9 Summary of Sediment Triad Relationships

Site	Arm of Triad		
	C-T	T-B	C-B
Dome Mine	√	√	X
Mattabi Mine	X	X	P
Myra Falls	√	P	√

√ = significant correlation
X = no correlation
P = partially effective

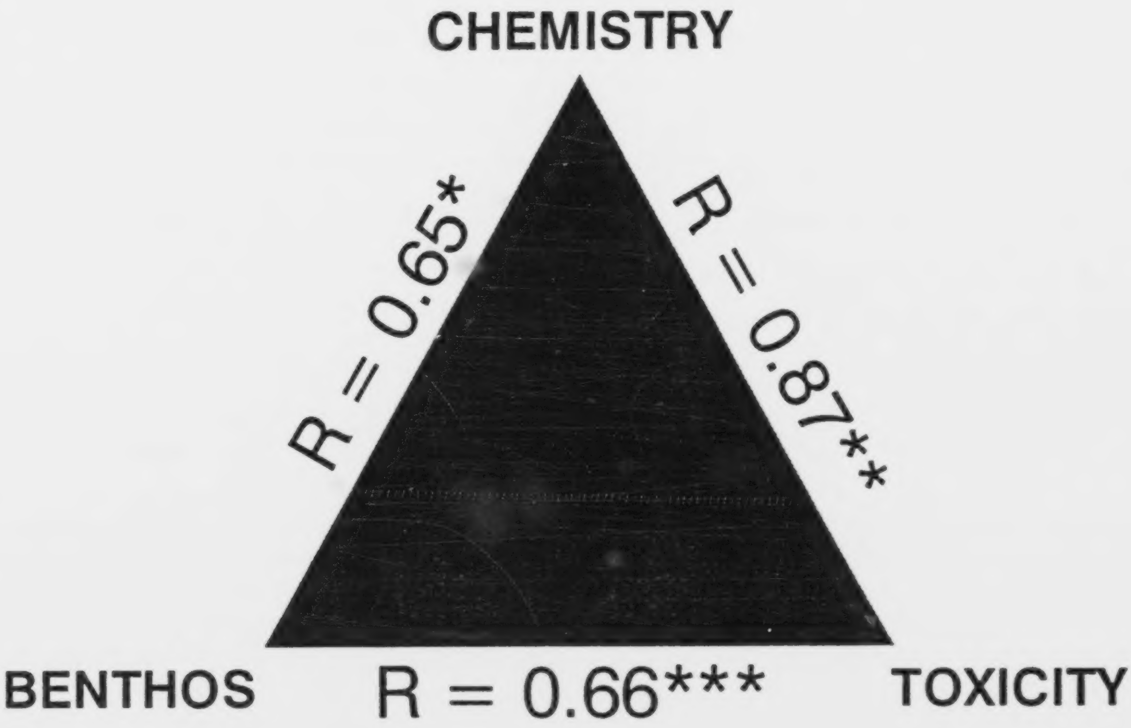
The significance of the parameter (C.T.B) correlations summarized in Table 7.9 are based on the dominant gradients for each parameter, in other words, the correlations that are mine related. The relationships were considered significantly correlated in 6 of the 9 possible cases. The triad relationships were not significant at 2 of the 3 situations at Mattabi. This is not surprising given there were few measurable biological effects at Mattabi.

There were many other examples of statistically significant correlations among secondary parameter gradients. These are not likely mine-related but do reflect other relationships in the environment. Discriminating between these correlations is necessary for data interpretation (BEAK/Golder 1998c).

Overall, the sediment quality triad was effective for identifying linkages between contaminants in sediments and biological responses. The sediment quality triad itself was not rigorously evaluated or considered as a monitoring tool. Therefore, there is no recommendation either way for its use in a routine monitoring program. However, its application for data interpretation and in a weight-of-evidence approach would be appropriate where data from each "arm" of the triad are available.

Pairwise comparison of the various individual toolboxes is considered a suitable procedure for data analysis and AETE supports this approach. However, AETE did not critically evaluate statistical methods using the same criteria as applied to the other tools.

Therefore, while AETE endorses the Sediment Quality Triad and pairwise comparison approaches, specific statistical methods cannot always be considered a routine monitoring tool, due to the dependence on site specific study design factors and the resultant availability of data.



Bartlett Sphericity Test = 37.36 ($p<0.001$)

*The relationship between sediment chemistry and the benthic community is statistically significant.

** The relationship between sediment chemistry and the toxicity tests (*Chironomus*, *Hyalella* and *Tubifex*) is statistically significant.

*** The relationship between the benthic community and the toxicity tests (*Chironomus*, *Hyalella* and *Tubifex*) is statistically significant.

Figure 7.2. Triad Approach to Evaluate Sediment Quality at Myra Falls

8. SUMMARY AND RECOMMENDATIONS

All tools considered in the AETE program were evaluated for potential use in a routine mine monitoring program. The primary criteria for evaluation included:

- must effectively measure an effect,
- must be a recognized tool with standard methods or protocols available,
- must be cost effective,
- must measure a relevant environmental variable,
- must be able to contribute to answering one or more of the four AETE guidance questions, and
- must contribute to testing of one or more of the thirteen AETE hypotheses.

The candidate tools endorsed for use in routine mine monitoring program are summarized in Table 8.1. It is recognized that the performance of individual monitoring tools did vary during the AETE program, and such variation can be expected during subsequent monitoring studies. Variability can be attributed to many confounding factors (e.g. habitat differences, different metal mixtures and bioavailability, different biological communities, etc) that must be considered when attempting to interpret results. However, the results of the AETE program do identify a suite of reliable tools that can be used in a monitoring program. Perhaps more importantly, the AETE program has provided methods for study design, data analysis and interpretation that can be used in a weight-of-evidence approach for assessing the aquatic effects of mine operations in Canada.

Table 8.1 Summary of Suitable Routine Monitoring Tools Recommended by the AETE Program

Question	Toolbox	Tool		Recommendation[1]	Report Section[2]
Are contaminants getting into the system?	Effluent Chemistry	1.1	Chemical parameters	Yes	4.1
	Effluent acute toxicity	2.1	*Daphnia* IQ test	No	4.2
		2.2	Microtox acute test	No	4.2
		2.3	Rototoxkit F test	No	4.2
		2.4	Thamnotoxkit F	No	4.2
		2.5	Toxichromotest	No	4.2
		2.6	Rainbow trout	Yes	4.2
		2.7	*Daphnia magna*	Yes	4.2
	Water Chemistry	3.1	Total metal concentrations	Yes	4.4
		3.2	Dissolved metal concentrations	No *	4.4
	Sediment Chemistry	4.1	Substrate mapping	No	4.5.1
		4.2	Surface sediment collection	Yes	4.5.1
		4.3	Sediment cores	No *	4.5.1
		4.4	Total metal concentrations	Yes	4.5.3
		4.5	Partial metal concentrations	No	4.5.3
		4.6	Porewater	No *	4.5.2
Are contaminants bioavailable?	Aquatic Plants	5.1	Metal concentrations	No	5.1.1

Table 8.1 Summary of Suitable Routine Monitoring Tools Recommended by the AETE Program

Question	Toolbox	Tool		Recommendation[1]	Report Section[2]
	Molluscs	6.1	Tissue metal concentrations	No *	5.1.2
	Fish Tissues	7.1	Organ tissue metal levels	Yes	5.1.3
		7.2	Tissue metallothionein levels	No	5.2
Is there a measureable response?	Effluent Sublethal Toxicity	8.1	Fathead minnow	Yes	6.1
		8.2	*Cenodaphnia dubia*	Yes	6.1
		8.3	*Selenastrum capricornutum*	Yes	6.1
		8.4	*Lemna minor*	Yes	6.1
		8.5	Algae multi-species test	No	6.1
		8.6	Microtox chronic	No	6.1
		8.7	Mutotox chronic	No	6.1
		8.8	Nematode survival	No	6.1
		8.9	Rainbow trout embryo	No	6.1
	Sediment Toxicity	9.1	*Chironomus riparius*	Yes	6.2
		9.2	*Hyalella azteca*	Yes	6.2
		9.3	*Tubifex tubifex*	No *	6.2
		9.4	Microtox solid phase	No	6.2
	Aquatic Plants	10.1	Phytoplankton community structure	No	6.3.1
		10.2	Periphyton community structure	No	6.3.1
		10.3	Macrophyte community structure	No	6.3.1
		10.4	Biochemical indicators	No	6.3.2
	Benthic Invertebrates	11.1	Mollusc growth	No	6.4.2
		11.2	Fitness parameters	No	6.4.1
		11.3	Community composition	Yes	6.4.3
		11.4	Artificial substrates	No	6.4.3
		11.5	Rapid assessment procedures	No	6.4.3
	Fish Survey	12.1	Biochemical indicators	No	6.5.1
		12.2	Histopathology	NR	6.5.2
		12.3	Fish organ size	Yes	6.5.3
		12.4	Fish growth	Yes	6.5.3
		12.5	Abundance indicators	Yes	6.5.3
		12.6	Community indicators	No *	6.5.3
Are contaminants causing this response?	Pairwise comparisons of the above toolboxes	13.1	Chemistry vs. biology correlations	Yes +	7.0
		13.2	Toxicity vs. biology	Yes +	7.0
		13.3	Chemistry vs. toxicity	Yes +	7.0

1. Yes = recommended as a suitable tool for routine monitoring
   No = not recommended as a suitable tool for routine monitoring
   No * = not recommended for routine monitoring but may have application in more detailed site specific investigations
   Yes + = the concept of pairwise comparisons and Sediment Quality Triad is supported by AETE where the necessary data are available and adequate, but they are not considered monitoring tools
   NR = no recommendation
2. Section of this report where tool is discussed.

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APPENDIX A

AETE PROGRAM MEMBERS

Aquatic Effects Technology Evaluation Program (1994-1998)
List of Committee Members, Collaborators and Participants:

Martin Barnett, Indian Affairs and Northern Development (MC)*
Al Beck, Manitoba Environment (TC)**
Brian Bell, Inco Limited (MC)
Norman Bermingham, Environment Canada (TC)
Vern Betts, Homestake (TC)
Willam Blakeman, Environment Canada (TC)
Marcia Blanchette, Natural Resources Canada (CANMET) (TC)
Hans Boerger, Syncrude (TC)
Peter Campbell, Institut National de Recherche Scientifique-Eau (TC)
Diane Campbell, Natural Resources Canada (CANMET) (TC- Chair)
William Coker, Natural Resources Canada (TC)
Ron Connell, Placer Dome Canada (TC)
Dick Cowan, Ontario Ministry of Northern Development and Mines (MC)
Daniel Cyr, Fisheries & Oceans (TC)
Kristin Day, Environment Canada (TC)
Kenneth Doe, Environment Canada (TC)
Ken Domine, Ministry of the Environment for Newfoundland & Labrador (MC)
Murray Duke, Natural Resources Canada (Geological Survey of Canada) (MC)
Charles Dumaresq, Environment Canada (TC)
William Duncan, Cominco Limited (TC)
Michel Filion, Teck Corporation (MC)
Gordon Ford, British Columbia Ministry of Environment, Lands and Parks (MC)
Sheila Forsyth, Environment Canada (TC)
Joe Fyfe, Falconbridge Limited (TC)
Elizabeth Gardiner, Mining Association of Canada (MC, TC)
Tom Gates, Saskatchewan Environment and Resource Management (MC)
Charles Gobeil, Fisheries & Oceans (TC)
Dave Green, Manitoba Environment (TC)
Roger Green, University of Western Ontario (TC)
Gwendy Hall, Natural Resources Canada (Geological Survey of Canada) (TC)
Landis Hare, Institut National de Recherche Scientifique-eau (TC)
Robert Hargreaves, Natural Resources Canada (CANMET)(MC)
Joseph Hubert, Université de Montréal (TC)
Peter Hulsman, Ontario Ministry of Natural Resources (TC)
Susan Humphrey, Environment Canada (TC)
Carolyn Hunt, Inco Limited (MC, TC)
Thomas Hynes, Natural Resources Canada (CANMET) (MC)
Irwin Itzkovitch, Noranda Technology Centre (MC)
Karen Keenleyside , Environment Canada (TC)
Bill Keller, Ministry of Northern Development and Mines (TC)
Brian Kett, Teck Corporation (TC)
Jack Klaverkamp, Department of Fisheries & Oceans (TC)
Elaine Koren, Environment Canada (TC)
Mark Liskowich, Saskatchewan Environment & Resource Management (TC)

Connie MacDonald, Environment Canada (MC)
Mike MacKinnon, Syncrude Research (TC)
Derrick Maddocks, Ministry of the Environment for Newfoundland & Labrador (MC)
Dave Madeley, Teck Corporation (TC)
Karen Mailhiot, Environment Canada (TC)
John Martschuk, Barrick Gold Resources Corporation (MC)
Bernard Matlock, Nova Scotia Environment (TC)
David Mchaina, Westmin Resources (TC)
Jacques McMullen, American Barrick Corporation (MC)
Bob Michelutti, Falconbridge (MC)
Kate Moir, Government of the Environment of Nova Scotia (TC)
Kelly Munkittrick, Environment Canada (MC, TC)
William Napier, Homestake (MC, TC)
Andrew Oliver, Natural Resources Canada (CANMET) (MC)
Roy Parker, Environment Canada (TC)
Joanne Parrott, Environment Canada (TC)
Jocelyne Pellerin-Massicotte, Université du Québec à Rimouski (TC)
Jeanne Percival, Natural Resources Canada (Geological Survey of Canada) (TC)
Bernadette Pinel Alloul, Université du Québec à Montréal (TC)
Larry Pommen, British Columbia Ministry of Environment (TC)
Robert Prairic, Noranda Technology Centre (MC, TC)
Patricia Rasmussen, Natural Resources Canada (Geological Survey of Canada) (TC)
Gary Rawn, Fisheries & Oceans (MC)
Alan Redenback, Environment Canada (TC)
Trefor Reynoldson, Environment Canada (TC)
Francine Richard, Ministère de l'environnement du Québec
Derek Riehm, Teck Corporation (MC, TC)
Robert Roy, Department of Fisheries and Oceans (TC)
Richard Scroggins, Environment Canada (MC, TC)
Ian Sharpe, B.C. Environment (MC, TC)
William Shilts, Natural Resources Canada (MC)
Julia Beatty-Spence, Ministry of the Environment for British Columbia (TC)
Michael Sprague, Ministry of the Environment for New Brunswick (MC)
André Tessier, Institut National de Recherche Scientifique-eau (TC)
Graham Van Aggelen, Environment Canada (TC)
Greg Vogelsang, Saskatchewan Environment & Resource Management (TC)
Allen Waroway, Indian Affairs & Northern Development (TC)
Glen Watson, Inco Ltd (TC)
Gary Westlake, Ontario Ministry of Environment & Energy (MC, TC)
Dwight Williamson, Manitoba Environment (TC)
Ken Yuen, Department of Fisheries and Oceans (MC)

***MC: Management Committee**
****TC: Technical Committee**

AETE Secretariat (Natural Resources Canada - CANMET):
Geneviève Béchard
Diane Campbell
Karen Mailhiot
Jennifer Nadeau
Joanne Papineau
Danielle Rodrigue
Madona Skaff
Lise Trudel

Collaborators for the Field Study Programs (1995-1997):

Martin Archambault, Agnico-Eagle (Mine Dumagami)
Brian Bell, Inco Limited
Victor Chapados, Noranda Mining and Exploration Inc.
Ron Connell, Placer Dome Canada
Joe Fyfe, Falconbridge
Robert Gardiner, Cominco Limited
David Honstein, Echo Bay Mines Limited
Carolyn Hunt, Inco Limited
Steven Januszewski, Westmin Limited
Réal Marcotte, Ministère des ressources naturelles du Qébec
Michael Patterson, Noranda Mining and Exploration Inc.
Philippe Poirier, Cambior (Mine Doyon)
Pierre Primeau, Barrick Gold (Complexe Bousquet)
Zoe Ramdin, Cominco Limited
Al Scott, Noranda Mining and Exploration Inc.

Toxicity testing program:

The Secretariat would like to extend their appreciation to all the mine personnel who collaborated in the toxicity testing program by graciously providing samples of mine effluents.

Consultants:

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APPENDIX B

PRELIMINARY LIST OF CANDIDATE TOOLS CONSIDERED BY AETE

Preliminary List of Candidate Monitoring Tools[1]

Sediment Sampling: Sub bottom acoustic profiling Grab and core sampling Sediment traps Chain of continuous documentation	Frigid finger Core subsampling Sediment preservation and processing Volumes required for laboratory analyses
Sediment Analysis: Particle size Bulk and clay mineralogy Specific gravity Moisture content Cation exchange capacity	Sequential leaching Whole sediment analysis XRF, ICP-ES(MS) Specific elements Organic and inorganic carbon & sulphur
Sediment Toxicity: Not addressed at this time	
Water Sampling: Depth integrators Grab samplers Automatic samplers Volume required for analyses	Preparation Containers Preservation and processing
Water Analysis: In situ measurements pH, Eh, temperature, turbidity, dissolved oxygen	Laboratory analyses Metals (As, Cu, Ni, Zn, Pb), acidity, alkalinity, hardness Major cations and anions

Benthos:

- Community level: (benthos community survey is focus)
  - Sampling devices
    - depositional sediments
    - erosional substrates
  - Mesh size 400-600 μm
  - Procedures
    - standard operating procedures
    - QA/QC
  - Taxonomic level of identification
    - identify highest possible level
  - Seasonality
    - preferably fall
  - Hydrological regime
    - look at historical hydrograph for low flow time
  - Control/upstream site
- Organism level: Look at literature for mouth part deformation than make decision.
- Tissue/Subcellular level: Methods appear to be experimental

Plankton/Macrophytes/Bacteria: Metal levels in tissues

Fisheries

Chemistry level: Metal levels in tissues	Organism level: Size at age Fecundity Growth rates Condition factor Anomalies Parasites
Biochemistry level: Inhibitory/inducible proteins (enzymes) Lipid and carbohydrate indicators Indicators: Growth	Population level: Size Age structure Growth rate Recruitment

Preliminary List of Candidate Monitoring Tools[1]

Reproduction Immune system Genotoxicity Respiratory Ion status	Mortality rate CPUE
Tissue level: Liver size Gonad size Histology Thyroid histology Metal analysis Skeletal strength, composition, elasticity Spleen size Gills	Community level: IBI

[1] List developed at Technical Committee meetings May 25-26, 1994, Hull, Quebec and September 30, 1994, Toronto, Ontario

APPENDIX C

ONE PAGE TOOL BOX SUMMARIES

HEADER: Effluent Chemistry

METHOD NAME: Chemical Parameters

AETE REPORT REFERENCE: No Technical evaluation, see field reports.

PURPOSE: Samples of liquid effluent are collected at the point of discharge to the receiving environment for chemical characterization. Samples can be analyzed for specific parameters for routine compliance monitoring for provincial, federal or other regulations or permits (e.g. Certificates of Approval, MMLERs). Results of chemical analysis can be combined with discharge volumes to determine loading rates.

DESCRIPTION: A literature search based technical evaluation of measuring effluent chemistry was not completed for AETE, however, measuring effluent chemistry was completed as part of every field study. The parameters measured included total and dissolved metals, general chemistry (e.g. alkalinity, chloride, sulphate, hardness, conductivity, full cation and anion balance), total suspended solids, nutrients, ammonia and cyanide when appropriate. Some effluent constituents can be useful to help define the effluent mixing zone. If downstream concentrations are compared with the original effluent concentrations then the amount of dilution can be calculated. If the effluent is toxic, detailed effluent chemistry can be used to help identify the causes of toxicity.

LIMITATIONS: Effluents are often complex mixtures of chemical substances. It is difficult to directly relate toxicity or in-stream biological effects directly to effluent chemistry results. Effluent chemistry can change substantially with operational changes or dilution from runoff. Therefore, simple occasional grab samples may not accurately reflect loading.

COST: In the range of $50 to $175 per sample depending on the number of analytes.

Was Tool Tested in AETE Field Program? Yes, effluent samples collected during each field survey.

AETE RECOMMENDATION: Yes, analyzing effluents for detailed chemistry is recommended as a component of a routine mine monitoring program.

HEADER: Water Chemistry

METHOD NAME: Total and dissolved metal concentrations.

AETE REPORT REFERENCE: 3.1.2., 3.1.3 plus field reports

PURPOSE: Specific objectives were to: 1) examine the use of dissolved metal and total metal concentrations to predict the biological effects of mine effluents. 2) examine the relationship between biological effects and the analytical detection limits for both dissolved and total metals. 3) identify cost effective alternatives to analyses for levels of dissolved or total metals as a means of predicting bioavailability.

DESCRIPTION: Dissolved metals are separated from total metals by filtration (0.45 μm). The effect of filtration as a tool for separating the dissolved metal fraction was examined. Several factors (seven) that might potentially influence toxicity of metals in effluents discharged to receiving waters were also examined. The use of speciation (equilibrium) models to predict uptake of metal ions was evaluated and identified as a potentially useful tool in addition to current analytical methods (toxicity testing). The following methods were evaluated for measuring total and dissolved metals in water: induced coupled plasma (ICP), atomic absorption spectrometry (AAS), anodic stripping voltammetry (ASV), ion selective electrodes (ISE), ion exchange resins, ion chromatography (IC), and bioassays.

The relationship between total or dissolved metal concentrations and biological effects was investigated in some detail as part of the hypotheses testing study component. Empirical data from the 1997 field studies suggested there was no preference for total or dissolved metal levels for predicting biological effects.

LIMITATIONS: Sample collection and processing for dissolved metals is a potential source of contamination.

COST: Cost for ICP analyses ranges from $90-125 per sample for a multiple metal scan. Cost for AAS analyses ranges from $8-15 per metal per sample. Additional cost and effort for filtering samples.

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Analysis of total metals is recommended for routine monitoring programs.

HEADER: Sediment Monitoring

METHOD NAME: Substrate Mapping

AETE REPORT REFERENCE : 3.2.1

PURPOSE: To provide information on the physical characteristics of bottom sediments including depths for bathymetry mapping. Characteristics of the substrate is used for habitat mapping and selection of sampling sites for benthic invertebrate surveys and surface samples for geochemical analysis.

DESCRIPTION: Different techniques were used during the 1995 field survey to map lake bottom types. The equipment tested included a) side scan sonar, b) Echo sounder, and c) Sub-bottom accoustic profiler. All techniques utilize various frequency signals to map the substrate. Bottom type was verified by collecting grab samples with an Ekman grab. Sub-bottom profiling was very precise and is reported to be able to penetrate sediments. However, confirmatory sediment cores suggested that sub-bottom profiling was subject to misinterpretation. Side scan sonar requires a non-uniform distribution of lake sediment type to allow identification of sediment types.

LIMITATIONS: Both side scan sonar and sub-bottom acoustic profiling have high capital costs, or high rental costs. All techniques require some practice or experience for reliable interpretation of the results.

COST: varies depending on type of system. Detailed costs provided in report #3.2.1.

Was Tool Tested In AETE Field Program? Yes, during 1995 field program.

AETE RECOMMENDATION: High frequency echo sounders are recommended for substrate mapping in conjunction with grab samplers for habitat mapping. The other methods are not recommended.

HEADER: Sediment Chemistry

METHOD NAME: Surface Sediment Collection.

AETE REPORT REFERENCE: 3.2.1

PURPOSE: The purpose of this method is to provide information on the spatial distribution of geochemical elements in a lake basin when investigating the effects of natural and anthropogenic inputs of geochemical elements into the system. The purpose of using a regularly-spaced grid is to simplify and keep track of the sampling site location and to provide uniform coverage of the surveyed area.

DESCRIPTION:

1. Birge-Ekman Sampler

It is suitable for sampling fine-grained, soft sediments and a mixture of silt and sand. The sampler has to be used under low current conditions in order to penetrate the sediment in a perpendicular orientation.

2. Ponar Grab Sampler

This sampler is commonly used with a winch or a crane hoist due to its weight. However, the Petite Ponar grab sampler weighs only approximately 10 kg and samples an area of 15 X 15 cm and is preferable for hand line operations. Both samplers are suitable for collecting coarse and firm bottom sediments as well as soft sediments.

3. Others

Other grab samplers are mentioned but are not described due to the required lifting capacity for these samplers (150 to 400 kg).

LIMITATIONS: The grab sampling method does not provide any information on temporal variability of elements (vertical distribution); provides spotty indication of the spatial variation of sediment type.

COST: Wildco-Eckman - sampler : 207$ U.S., sampler & case : 275$ U.S., Wildco kit : 335$ U.S. (includes a line and a messenger); Petite Ponar - sampler 439$ U.S., sampler & case : 519$ U.S., line (100 ft, 316' polyester) : 23.50$ U.S. Box Corer - sampler, line, extra weight : 547$.

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Collection of surface sediments is recommended for geochemical analysis. The most suitable grab-sampling device is the Petite Ponar with the addition of a release mechanism to increase its sampling flexibility.

HEADER: Sediment Chemistry

METHOD NAME: Sediment Core Sampling.

AETE REPORT REFERENCE: 3.2.2.

PURPOSE: To determine the natural background concentrations of metals in order to quantify anthropogenic contamination due to mining. Core data can be compared with surficial sediment quality data to determine the depth to which contaminated sediments have accumulated. Also, rate of sedimentation can be determined in conjunction with dating of sediments.

DESCRIPTION: Core samplers are a common sampling device used to sample bottom sediments in aquatic environments. Detailed profiles of sediment types and chemical composition can be obtained by analyzing individual sediment layers.

A number of sediment core samplers are available and the details of each are described in the AETE report 3.2.2 (sample length and diameter, how the sampler works, etc.).

Sample processing is dependent on the type and number of analyses required, however subsampling into layers should be completed as soon as possible. Sediment cores collected for chemical analyses are usually subsampled into 1 cm sections. Initial analysis should be completed on every third subsample, retaining the remaining samples for analyses if required.

LIMITATIONS: Core samplers are generally designed to sample fine-grain sediments. Migration of metals within the sediment column cannot be determined. There is some spatial variation of natural metal concentrations.

COST: Sample Analysis: $125 - 150 per sub-sample (includes metal scan via ICP-MS, mercury, sulphur and anions). Total cost dependent on: level of detail of subsampling (e.g. 1 cm vs. 3 cm increments), no. of subsamples analyzed, cost of sampling gear, and travel expenses.

Was Tool Tested in AETE Field Program? Yes (1995 program).

AETE RECOMMENDATION: Core samples are not recommended for routine monitoring. They may be suitable for more detailed monitoring to provide rates of deposition and background concentrations on a site specific basis.

HEADER: Sediment Chemistry

METHOD NAME: Total metal concentrations in sediments

AETE REPORT REFERENCE: 3.2.2

PURPOSE: To review analytical methods available for the determination of total metal concentrations in sediments.

DESCRIPTION: Many sediment quality guidelines are currently based on total metal concentrations in sediments. Total, or bulk, sediment chemistry provides information on the loading rates of particular elements and depositional patterns. Techniques available for determination of metals in sediments include atomic absorption spectrophotometry (AAS), X-ray fluorescence (XRF), instrumental neutron activation analysis (INAA), inductively coupled atomic absorption spectrophotometry (ICP-AES) and ICP-mass spectrometry (ICP-MS).

The concentrations of metals in sediments, particularly in mining areas are generally relatively high such that sample contamination from handling and sample containers is not a concern. Secondly, analytical techniques with higher detection limits (e.g. ICP-AES) are generally sufficient at lower cost per sample.

Bulk samples are digested using either *aqua regia* or a mixture of perchloric, nitric and hydrochloric acids for extraction of total metals. Metal concentration can be influenced by sediment particle size and organic carbon content. These parameters should be measured in sediments simultaneously and metal content normalized if necessary.

LIMITATIONS: Total metal concentrations may not be directly related to biological availability and toxicity.

COST: depends on number of parameters analyzed and technique used. Single elements $10-$20, multi element scans up to $175. Particle size and organic carbon $40 to $75.

Was Tool Tested In AETE Field Program? Yes

AETE RECOMMENDATION: Analysis for total metals is recommended as a suitable tool for a routine mine monitoring program.

HEADER: Sediment Chemistry

METHOD NAME: Partial Metal Extraction

AETE REPORT REFERENCE: 3.2.2 plus 1997 site reports.

PURPOSE: To review procedures for partial extraction of metals from sediments for analysis of specific chemical forms of metals.

DESCRIPTION: It is generally thought that a particular chemical form of an element determines its behavior, biological availability and potential toxicity rather than the total concentration in sediments. Specific chemical forms can be measured a) by direct instrument techniques, b) directly by sequential digestion of sediments, or c) indirectly by predicting levels through thermodynamic modelling.
Direct instrument techniques include X-ray photoelectron spectrometry (XPS), scanning electron microscopy/X-ray microanalysis (SEM/XRM); secondary ion mass spectrometry (SIMS) and Auger electron spectrometry (AES). These methods have been applied to geochemical studies and for mineral exploration.

The relative strength of association between metals and particles can be assessed by single or sequential extraction or sediment digestion methods. Weak acids or chelating agents (e.g. EDTA) and reducing agents may be used to differentiate between different chemical forms. Sediment fractions can be operationally defined (e.g. ferromanganese oxyhydrides) depending on the digest method used. The recent acid volatile sulphide (AVS) concept assumes that metal concentrations in porewater of anoxic sediments are controlled by sulphides. AVS are extracted by cold-acid purge and trap technique. Simultaneously extracted metals (SEM) represent the portion of total metals released during AVS dissolution. The SEM/AVS ratio is sometimes used to characterize metal availability.

Samples of extract are analyzed by similar analytical techniques as for total metals (see Toolbox Summary #3.4).

LIMITATIONS: Anoxic sediment samples must be carefully collected and stored to prevent oxidation. Partial extraction and AVS procedures are not routine for most labs and are relatively expensive. During the AETE field program there was no relationship between sediment toxicity and SEM/AVS ratio.

COST: will vary substantially from approximately $20 for single metal/digest analysis to several hundred dollars for full total metal and SEM/AVS determination.

Was Tool Tested In AETE Field Program? Yes

AETE RECOMMENDATION: Partial extraction of sediments is not recommended for a routine monitoring program.

HEADER: Sediment Monitoring

METHOD NAME: Sediment Porewater Sampling and Chemistry

AETE REPORT REFERENCE: 3.2.2

PURPOSE: To select a proper technique for collection of sediment porewater. Porewater acts as a link between bottom sediments and the overlying water. The analysis of porewater can provide information on geochemical changes in the sediment and availability of metals to biota. Porewater samples are being increasingly used for chemical analysis and for toxicity testing purposes.

DESCRIPTION: Sediment porewater or interstitial water, is the water filling the spaces between sediment particles. With the exception of the sediment-water interface, sediments and associated porewater are generally anoxic. Therefore, collection techniques must be rapid and maintain oxygen free conditions to prevent changes in chemical speciation. Porewater samples can be collected a) indirectly or b) directly. Indirect techniques involve recovering porewater from previously collected sediment samples. Samples are squeezed, centrifuged and filtered. Direct sampling involves *in situ* collection. Direct suction of porewater using a variety of filters of ceramic, teflon, paper or glass fibre or syringes was the original collection technique. More recently, porewater samples have been collected using dialysis across membranes based on diffusion transport. Samplers using membrane dialysis are also known as "peepers" and are probably the most common technique for collecting samples for chemical analysis.

LIMITATIONS: *In situ* methods (e.g. peepers) generally only collect very small volumes of water not sufficient for biological testing. Peepers are preferred for chemical sampling. Samples have very short storage times.

COST: not provided

Was Tool Tested In AETE Field Program? No

AETE RECOMMENDATION: Collection of porewater for either chemistry or toxicity analysis is not recommended for a routine mine monitoring program. However, these methods may provide useful detailed information in site specific studies.

HEADER: Alternative Acute Toxicity Tests: Effluent Toxicity Testing

METHOD NAME: *Daphnia* IQ Test

AETE REPORT REFERENCE: 1.1.1, 1.1.4

PURPOSE: Proposed as rapid alternative to trout and *Daphnia* acute lethality tests. The objective is to determine the concentration of effluent that causes a 50% reduction in fluorescence, compared to control organisms, after addition of an additive. The result is expressed as an effective concentration for 50% of the population (EC50). The test results can be used to answer the question "Is there a measurable response?"

DESCRIPTION: The *Daphnia* IQ test is a self-contained test system. The test is described in the American Society of Testing and Materials publication ASTM E-47, proposal P235 "Proposed test method for fluorometric determination of toxicity induced enzymatic inhibition in *Daphnia magna*".

Daphnids, ranging in age from two to five days old, are exposed to effluent concentrations for a 1 hour period, after which the substrate is added to the exposure chambers. After being taken up, the substrate is transformed into a fluorescent compound. After a 15 minute incubation, the chambers are illuminated with ultraviolet light and the number of fluorescent animals is estimated. A reduction in fluorescence compared to controls indicates toxicity.

The laboratory should maintain detailed records of all aspects of the samples, test organisms, culture maintenance, test conditions, equipment and test results. There is no standardized QA/QC program to ensure the performance of the batch of test organisms.

Test requirements include a temperature controlled room, invertebrate culture facilities and an ultraviolet light source. Technical personnel must be skilled in bioassays.

LIMITATIONS: The IQ test is more sensitive than the trout test and may overestimate effluent toxicity. There is no standardized QA/QC program. Endpoints are determined by visual observation and the result may be subjective.

COST: Not routinely performed by commercial laboratories, kit costs $70

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Not recommended for routine monitoring since standardized protocol and QA/QC are required.

HEADER: Alternative Acute Toxicity Tests: Effluent Toxicity Testing

METHOD NAME: Microtox Acute Test

AETE REPORT REFERENCE: 1.1.1, 1.1.4

PURPOSE: Considered as an alternative to the rainbow trout test. To evaluate effects of effluent exposure on light production by the naturally luminescent marine bacteria, *Vibrio fischeri*. The result is expressed as the concentration where light output is reduced by 25% or 50% (IC25, IC50)

DESCRIPTION: The Microtox test is frequently used on site at many industries. The test is described in the manufacturer's handbook "Microtox Manual. A Toxicity Testing Handbook." (Microbics Corp. 1992).

The Microtox test is a rapid screening bioassay kit, which measures toxic effects on the light output of a standardized luminescent bacterial culture. The light output of the bacteria is measured after 5 or 15 minutes exposure to the sample. This is a rapid assay since the duration of the entire test is only 30 minutes.

For QA/QC, detailed records of all aspects of the samples, test organisms, test conditions, equipment and test results are validated and kept by the laboratory. Reference toxicant tests are performed at the same time as samples are tested to validate the performance of the batch of test organisms.

LIMITATIONS: The test organism is a marine bacteria, with little relevance to Canadian mining environments.

COST: $280

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: This test was not sensitive to mine effluent and it is not recommended as an alternative to the rainbow trout test in routine monitoring.

HEADER: Alternative Acute Toxicity Tests: Effluent Toxicity Testing

METHOD NAME: Rototoxkit F, Test with Freshwater Rotifer *(Brachionus calyciflorus)*

AETE REPORT REFERENCE: 1.1.2, 1.1.4

PURPOSE: To be used as a rapid alternative to trout and *Daphnia* acute lethality tests. The purpose is to determine the concentration of effluent that causes 50% mortality after a 24 hour exposure. The result is expressed as a lethal concentration for 50% of the population (LC50). The test results can be used to answer the question "Is there a measurable response?"

DESCRIPTION: Rotifers are members of the zooplankton community which commonly occur in lakes and streams and are a source of food for larval and adult fish. Rototox F is a test kit which includes rotifer cysts and all equipment needed for the test. The test is described in the manufacturer's Standard Operating Procedure "Rototox F: Rotifer toxicity screening test for freshwater" (Creasel Ltd.).

The cysts are incubated for 24 hours prior to the start of the test. Most cysts hatch 20 hours later and the neonates are transferred onto microplate wells containing the effluent exposures. These are prepared in a standard freshwater. After 24 hours the numbers of live, dead and immobilized neonates are counted under a microscope.

For QA/QC, a reference toxicant is tested at the same time as an effluent sample. Successive reference toxicant data are plotted on a control chart. If results are within expected limits, the performance of the batch of test organisms is ensured.

The organisms can be stored as cysts in the dark at 6°C and remain viable for up to one year. Organisms are hatched one day prior to initiation of testing, eliminating the time and expense of culturing and maintaining the test organisms. Test requirements include a temperature controlled room and a microscope. The required test tubes, test wells, pipettes, hatching and dilution media, and rotifer cysts are provided by the manufacturer. Technical personnel must be skilled in bioassays.

LIMITATIONS: The test is relatively recent and has a low sensitivity to mining effluents. There is a lack of standardized QA/QC requirements.

COST: Not routinely performed by commercial laboratories, kit costs $45

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Not recommended as a suitable tool in routine monitoring.

HEADER: Alternative Acute Toxicity Tests: Effluent Toxicity Testing

METHOD NAME: Thamnotoxkit F. Tests With Freshwater Crustaceans *(Thamnocephalus platyurus)*

AETE REPORT REFERENCE: 1.1.2, 1.1.4

PURPOSE: To be used as a rapid alternative to trout and *Daphnia* acute lethality tests. The purpose is to determine the concentration of effluent that causes 50% mortality after a 24 hour exposure. The result is expressed as a lethal concentration for 50% of the population (LC50). The test results can be used to answer the question "Is there a measurable response?"

DESCRIPTION: Thamnotoxkit F is a cyst-based toxicity test using the fairy shrimp *(Thamnocephalus platyurus)*. Fairy shrimp have a wide geographical distribution and are representative of the crustacean zooplankton community. The test is described in the manufacturer's Standard Operating Procedure "Thamnotoxkit F: Crustacean toxicity screening test for freshwater" (Creasel Ltd.).

The Thamnotox cysts are incubated for 24 hours before testing. Within 2-4 hours after hatching, the larvae are transferred into microplate test wells containing the effluent exposures. The effluent concentrations are prepared with a standard freshwater. After 24 hours of incubation in darkness, the numbers of live and dead neonates are counted under a microscope.

For QA/QC, a reference toxicant is tested at the same time as an effluent sample. Successive reference toxicant data are plotted on a control chart. If results are within expected limits, the performance of the batch of test organisms is ensured.

Test requirements include a temperature controlled room and a microscope. Technical personnel must be skilled in bioassays.

LIMITATIONS: There is a lack of standardized QA/QC requirements.

COST: Not routinely performed by commercial laboratories, kit costs $45.

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Not recommended as a suitable tool in routine monitoring.

HEADER: Alternative Acute Toxicity Tests: Effluent Toxicity Testing

METHOD NAME: Toxi-chromotest

AETE REPORT REFERENCE: 1.1.2, 1.1.4

PURPOSE: To be used as a rapid alternative to trout and *Daphnia* acute lethality tests. The purpose is to determine the concentration of effluent that reduces the production of a bacterial enzyme important for growth. The result is expressed as a Minimal Inhibitory Concentration (MIC), defined as the concentration of a chemical causing a 20% reduction. The test is described in the manufacturer's handbook "The toxi-chromotest" (Environmental Bio Detection Inc., 1993).

DESCRIPTION: The toxi-chromotest uses a colorimetric endpoint to estimate the concentration of sample that causes 20% toxicity to an engineered strain of *E. coli* after a 90 minute incubation. The test involves a genetically engineered strain of the bacteria *Escherichia coli* (strain K12 OR85) and an inducible enzyme (b-galactosidase) which is produced when the bacteria is functioning normally. The bacteria are exposed to a solution containing an inducer of the enzyme b-galactosidase. The activity of the enzyme is detected by the hydrolysis of a chromogenic color substrate. Toxic substances interfere with the recovery process and subsequently with the synthesis of the enzyme and the color reaction. The bacteria are exposed to an effluent for a period of 90 minutes. If a sample is toxic, no enzyme is synthesized and no color develops. If the sample is non-toxic, the enzyme is synthesized and a distinct blue colour develops.

For QA/QC, a reference toxicant is tested at the same time as an effluent sample. Successive reference toxicant data are plotted on a control chart. If results are within expected limits, the performance of the batch of test organisms is ensured.

Test requirements include a temperature controlled incubator and a spectrometer. Technical personnel must be skilled in bioassays.

LIMITATIONS: The test is relatively recent and has a low sensitivity to mining effluents. There is a lack of standardized QA/QC requirements.

COST: Not routinely performed by commercial laboratories, kit costs $38

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Not recommended as a suitable tool for routine monitoring.

HEADER: Alternative Acute Toxicity Tests

METHOD NAME: Rainbow Trout Acute Lethality Test

AETE REPORT REFERENCE : 1.1.2, 1.1.4,

PURPOSE: To determine the concentration of effluent that causes 50% mortality to rainbow trout during a 96 hour exposure period.

DESCRIPTION: This standard tests involves placing groups of 10 fish per treatment in a range of effluent concentrations. Effluent is diluted with freshwater to which the fish have been acclimated. Tests are conducted at 15 ± 1°C. Both temperature and photoperiod are similar to culture conditions. Solutions are gently aerated throughout the exposure period. Tests are conducted under static conditions with no renewal of the test solutions. Observations for immobility or mortality are recorded at 24, 48, 72 and 96 hours. A fish is considered dead if there is no evidence of opercular activity or no response to gentle prodding. The rainbow trout acute lethality test is described in detail in the Federal Protocol (Environment Canada 1992).

This test is widely used and accepted as a regulatory test throughout Canada.

LIMITATIONS: Rainbow trout may not be native to all receiving waters.

COST: Approximately $350

Was Tool Tested In AETE Program? Yes

AETE RECOMMENDATION: This widely recognized test is recommended as a suitable tool for routine effluent testing.

HEADER: Alternative Acute Toxicity Tests

METHOD NAME: *Daphnia magna* Acute Lethality Test

AETE REPORT REFERENCE : 1.1.2, 1.1.4,

PURPOSE: To determine the concentration of effluent that causes 50% mortality to *Daphnia magna* during a 48 hour exposure period.

DESCRIPTION: This standard tests involves placing groups of < 24 hour old *D. magna* neonates into a range of effluent concentrations. Effluent is diluted with freshwater to which the invertebrates have been acclimated. Toxicity tests are conducted in test tubes, generally 55 mL in size. For each concentration, including controls, 4 replicate tubes are set up each with 3 daphnids for a total of 12 individuals per concentration. Tests are conducted at 20 ± 1°C. Both temperature and photoperiod are similar to culture conditions. Tests are conducted under static conditions with no renewal of the test solutions. Observations for immobility or mortality are recorded at 24 and 48 hours. A daphnid is considered dead if there is no visible heartbeat upon microscopic examination. The *Daphnia magna* lethality test is described in detail in the Federal Protocol (Environment Canada 1990).

This test is widely used and accepted as a regulatory test throughout Canada.

LIMITATIONS: *Daphnia magna* are not native to all receiving waters.

COST: Approximately $250

Was Tool Tested In AETE Program? Yes

AETE RECOMMENDATION: This widely recognized test is recommended as a suitable tool for routine effluent testing.

HEADER: Biological Monitoring

METHOD NAME: Metal levels in plant tissues

AETE REPORT REFERENCE: 2.3.2, 1997 Field study at Heath Steele

PURPOSE: To determine bioavailability of metals in the environment by measuring metal levels in plant tissues.

DESCRIPTION: The concentration of metals can be measured in different groups of aquatic plants including phytoplankton, periphyton and macrophytes. For larger plants, different tissues are analyzed including leaves, stems and roots. Metal concentrations will vary with tissue sampled as well as seasonally. Samples collected in the field can be frozen or freeze dried for preservation for analysis. Chemical analysis involves tissue digestion followed by routine procedures depending upon parameter being measured.

LIMITATIONS: There is no standardized protocol for this method. Considerable variability may be observed in the data due to biological factors that are not fully understood. The relationship between metal levels in plants and ambient metal loading has not been clearly demonstrated.

COST: Chemical analysis will vary depending on parameters measured: $25 to $175

Was Tool Tested In AETE Program? Yes, metals in periphyton were measured at Heath Steele in 1997.

AETE REPORT RECOMMENDATION: This tool is not recommended for routine monitoring of mining effects.

HEADER: Biological Monitoring

METHOD NAME: Metal levels in molluscs

AETE REPORT REFERENCE : 2.3.1

PURPOSE: To evaluate the use of molluscs as "sentinel species" to measure contaminant concentrations in aquatic ecosystems through the accumulation of contaminants in their tissues.

DESCRIPTION: Molluscs are used as bioaccumulative biomonitors, also called "sentinel species", to measure contaminant concentrations in aquatic ecosystems through the accumulation of contaminants in their tissues. Sentinel molluscs are used to determine the relative degree of loading and spatial extent of metal contamination.

LIMITATIONS: The relationships between mollusc tissue metal and metallothionein concentrations and effects at the individual, population, and ecosystem level are not well established. Availability of molluscs for sampling and collection needs to be established before incorporating them into a long-term national biomonitoring program. Numerous abiotic and biotic measurements must be made in conjunction with metal concentrations in molluscs to interpret effectively the field results. Metal-induced effects in molluscs such as MT concentrations are not well established and these responses should be used with caution.

COST: Costs are associated with animal collection, beginning and end-of-test measurements, deployment and retrieval activities, and chemical analyses. Total costs for a pilot project ranged from $16,000 U.S. to $40,000 U.S. depending on the study team. These costs do not include travel and are dependent on the remoteness of collection and retrieval sites.

Was Tool Tested in AETE Field Program? No.

AETE RECOMMENDATION: Metal levels in molluscs may be used in conjunction with other organisms (e.g. fish, invertebrates, plants) to monitor metal concentrations on a site specific basis, but are not recommended as a stand alone tool for routine monitoring.

HEADER: Biological Monitoring

METHOD NAME: Fish Tissue Metal Levels.

AETE REPORT REFERENCE: 2.2.3

PURPOSE: To determine metal levels in fish tissue as a useful indicator of effects due to mining operations.

DESCRIPTION: The concentrations of metals are measured in fish tissues including whole fish for smaller specimens (<10 cm). This methodology has been used extensively for environmental assessments and QA/QC measures are well established. Most analyses are widely commercially available. Earlier studies generally only used muscle tissue. However, only mercury analysis in muscle is considered useful. Other metals (e.g. Cu, Zn, As) should be measured in liver, kidney or gill. Kidney tissues can be difficult to obtain in some species. Analytical method used is important for some elements (e.g. Se, As, Hg) to achieve proper detection limits. Tissue metal levels can be modified by species, age, sex and size so these variables should be standardized between collection areas for meaningful comparison of results. A minimum sample size of 5-10 per tissue type for each area is generally recommended.

LIMITATIONS: Many metals are essential elements (e.g. Zn, Cu, Ni), therefore, concentrations in tissues are homeostatically regulated to some degree and tissue concentrations will not be in direct proportion to environmental loading. "Background" concentrations for some metals (e.g. Zn) can vary widely. Whole body analyses may have interference from gut contents. Tissue metals have not been widely correlated with biological effects. Therefore, metal levels provide a good indicator of exposure but not biological impacts.

COST: Ranges from approximately $20 per sample for single metal analyses, to about $200 for multi-element scans.

Was Tool Tested In AETE Field Program? Yes, all 3 years.

AETE RECOMMENDATION: Measurement of metals in muscle and liver in large fish, and viscera of small fish are recommended as suitable tools for environmental exposure in routine monitoring. Gill and kidney tissues may also be considered on a site specific basis.

HEADER: Biological Monitoring

METHOD NAME: Metallothionein in fish tissue

AETE REPORT REFERENCE: 2.2.1.

PURPOSE: Metallothionein (MT) is a protein considered as a biomarker for exposure to metals, primarly Zn, Cu, Cd, and Ag.

DESCRIPTION: MT are a low molecular weight metal-binding protein that have a high affinity for certain metals. Studies suggest they play a role in the regulation of essential metals such as Zn and Cu, and in the detoxification of nonessential metals such as Cd.

Samples collected in the field for MT analysis must be frozen on dry ice and transported to the laboratory frozen. Reliable analytical methods are known, but there is a need to standardize protocols for sample preparation, MT extraction and quantification in Canada. At present MT analysis is not routinely performed by any private laboratory, although this could be quickly developed. The analytical method requires sophisticated equipment which may include Liquid Chromatography (LC), High Performance LC (HPLC), Graphite Furnace - Atomic Absorption Spectrophotometry (GF-AAS) or Inductively Coupled Plasma (ICP) atomic absorption spectrometry or ICP mass spectrometry and radioimmunoassay techniques.

Standard Reference Material is available. Under good storage conditions, MT levels should be stable for months to years, however, repeated thawing and freezing will affect results. MT can be considered a useful biomarker for exposure to certain metals.

LIMITATIONS: Dose-response curves between metal exposure and MT levels are not well established. Biological factors including reproductive status of the animal can affect MT levels. These factors do not appear to be well documented. The use of MT levels as a measure of effect to either the individual or population is not well established.

COST: Estimated commercial cost is $40 - $70 per sample.

Was Tool Tested In AETE Field Program? Yes

AETE RECOMMENDATION: MT is not recommended as a suitable tool for a routine mine monitoring program.

HEADER: Effluent Toxicity Testing: Sublethal Toxicity Testing.

METHOD NAME: Growth and survival of larval fathead minnows.

AETE REPORT REFERENCES: 1.2.1, 1.2.2, 4.1.2

PURPOSE: Evaluate effects of effluent exposure to an early life stage of fish. Result is expressed as the concentration where larval growth/survival is reduced by 25% (IC25). If mortality is significant, it may be possible to calculate the lethal concentration for 50% of the test population (an LC50). The test results can be used to answer question "Is there a measurable response?"

DESCRIPTION: A widely used toxicity test in North America. The reference for the test method is Environment Canada EPS 1/RM/22. (Biological test method: test of larval growth and survival using fathead minnows Environment Canada. 1992).

Fathead minnow larvae, less than 24 h old, are exposed to a minimum of five effluent concentrations and laboratory water control. At the conclusion of the test, surviving fish in each beaker are counted and weighed.

For QA/QC, detailed records of all aspects of the samples, test organisms, culture maintenance, test conditions, equipment and test results are validated and kept by the laboratory. A reference toxicant is used to establish the validity of effluent toxicity data. Successive reference toxicant data are plotted on a control chart. If results are within expected limits, the performance of the batch of test organisms is ensured. Minimum level of data reporting is required as outlined in the test methods.

Test requirements include a temperature controlled room, toxicity testing equipment (exposure containers), fish culture facilities (since the test uses newly hatched larval fish) and a source of clean non-chlorinated water. Technical personnel must be skilled in fish culture and bioassays. The test requires approximately 40 L of effluent.

LIMITATIONS: Organisms may require an acclimation period if receiving water is to be used as control/dilution water. The test may be invalid if the receiving waters are even slightly toxic to the test organisms. However, pre-acclimation of test organisms may resolve control mortality problems resulting from the use of site waters as control/dilution water in the test. The test cannot be used in regions where the fathead minnow is not a native species (e.g., British Columbia, Northwest territories, Yukon, Newfoundland). The larval test was not as sensitive to mine effluent compared to other sublethal tests evaluated.

COST: $1077 (average from six CAEAL/MEF accredited labs -EEM Toxicology Expert Working Group/Final Report).

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Recommended as a suitable tool for routine monitoring.

HEADER: Effluent Toxicity Testing: Sublethal Toxicity Testing.

METHOD NAME: Test of reproduction and survival using the cladoceran *Ceriodaphnia dubia*.

AETE REPORT REFERENCE: 1.2.1, 1.2.2, 4.1.2

PURPOSE: Evaluate effects of effluent exposure on the reproduction of an invertebrate. Receiving water can be used as dilution/control water for the test. Result is expressed as the concentration where the average number of young per female is reduced by 25% (IC25). If mortality is significant, it may be possible to calculate the lethal concentration for 50% of the test population (an LC50). The test results can be used to answer the question "Is there a measurable response?"

DESCRIPTION: A widely used toxicity test in North America. The test method is described in Environment Canada EPS 1/RM/21. (Biological test method: test of reproduction and survival using the cladoceran *Ceriodaphnia dubia*. Environment Canada 1992).

Young ceriodaphnids, less than 24 h old, are exposed to a minimum of five effluent concentrations and a control. The test is completed when at least 60% of the surviving control organisms have had three broods of neonates (7 to 8 days). Survival of adults and number of young are recorded daily. At the end of the assay, the average number of young produced in each exposure concentration is calculated.

For QA/QC, detailed records of all aspects of the samples, test organisms, culture maintenance, test conditions, equipment and test results are validated and kept by the laboratory. A reference toxicant is used to establish the validity of effluent toxicity data. Successive reference toxicant data are plotted on a control chart. If results are within expected limits, the performance of the batch of test organisms is ensured. A minimum level of data reporting is required as outlined in the test methods.

Test requirements include a temperature controlled room, bioassay equipment (exposure containers), invertebrate culture facilities (since the test uses newly emerged neonates) and a source of clean non-chlorinated water. Technical personnel must be skilled in invertebrate culture and bioassays. The test requires approximately 3-4 L of effluent.

LIMITATIONS: The test may be invalid if the receiving waters are toxic to the test organisms. However, acclimation of test organisms may resolve any control toxicity resulting from the use of site waters as control/dilution water.

COST: $977 (average from six CAEAL/MEF accredited labs -EEM Toxicology Expert Working Group/Final Report).

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Recommended as a suitable tool for routine monitoring.

HEADER: Effluent Toxicity Testing: Sublethal Toxicity Testing

METHOD NAME: Growth inhibition of the alga *Selenastrum capricornutum*

AETE REPORT REFERENCE: 1.2.1, 1.2.2, 4.1.2

PURPOSE: Evaluate effects of effluent exposure on the growth of a unicellular freshwater alga. Result is expressed as the concentration where the number of cells is reduced by 25% (IC25). The test results can be used to answer the question "Is there a measurable response?"

DESCRIPTION: A microplate adaptation of the US EPA "Algal Assay Procedure". The test method is described in Environment Canada. EPS 1/RM/25 (Biological test method: growth inhibition test using the freshwater alga *Selenastrum capricornutum*. Environment Canada 1992).

The sample dilution and a small volume of a nutrient solution is inoculated with exponentially growing algal cells on a 96 well microplate. The microplate is incubated under constant illumination for 72 h. At the end of the assay, the microplate wells are mixed and the cells are counted.

For QA/QC, detailed records of all aspects of the samples, test organisms, culture maintenance, test conditions, equipment and test results are validated and kept by the laboratory. A reference toxicant is used to establish the validity of effluent toxicity data. Successive reference toxicant data are plotted on a control chart. If results are within expected limits, the performance of the batch of test organisms is ensured. A minimum level of data reporting is required as outlined in the test method.

Test requirements include an incubator with light and temperature control, a particle counter, algal culture facilities and a source of high quality deionized/distilled water. Technical personnel must be skilled in algal culture, axenic technique and bioassays. The *Selenastrum* test requires <1 L of effluent.

LIMITATIONS: The test may be invalid if the receiving waters are toxic. Effluents and receiving waters must be filtered (to remove bacteria and other algae) which may modify sample toxicity.

COST: $513: Average from six CAEAL/MEF accredited labs (EEM Toxicology Expert Working Group Final Report).

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Recommended as a suitable tool for routine monitoring.

HEADER: Effluent Toxicity Testing: Sublethal Toxicity Testing

METHOD NAME: Growth inhibition of the duckweed *Lemna minor*

AETE REPORT REFERENCE: 1.2.1, 1.2.2, 4.1.2

PURPOSE: Evaluate effects of effluent exposure on the growth of a freshwater plant. Result is expressed as the concentration where leaf production is reduced by 25% (IC25). Results are useful to answer the question "Is there a measurable response?"

DESCRIPTION: The freshwater duckweed plant grows in most regions of Canada. The test method is described in Environment Canada. EPS 1/RM/37 (Biological Test Method: Test for measuring the inhibition of growth using the macrophyte, *Lemna minor*).

Fast growing cultures of *Lemna minor* are exposed to concentrations of effluent under static conditions and constant illumination. Plants are acclimated to the test media for 24 h before testing. After 7 days, the number of leaves produced in each exposure concentration is counted. For a valid test, the number of leaves on control plants must increase by 10-fold at the end of the test.

For QA/QC, detailed records of all aspects of the samples, test organisms, culture maintenance, test conditions, equipment and test results are validated and kept by the laboratory. A reference toxicant test is used to establish the validity of effluent toxicity data. Successive reference toxicant data are plotted on a control chart. If results are within expected limits, the performance of the batch of test organisms is ensured. Minimum level of reporting is required as outlined in the test method.

Test requirements include an incubation chamber with controlled temperature and illumination, bioassay equipment, plant culture facilities and a source of clean non-chlorinated water. Technical personnel must be skilled in plant culture, axenic technique and bioassays. The test requires approximately 1-2 L of effluent.

LIMITATIONS: The test may be invalid if the receiving waters are toxic. However, acclimation techniques may resolve the problem. Effluents and receiving waters must be filtered before testing to remove algae. Environment Canada test method available. Canadian commercial laboratories are available to provide testing services for this test.

Was Tool Tested in AETE Field Program? Yes.

COST: $500 (approximately same as for *Selenastrum capricornutum*).

AETE RECOMMENDATION: Recommended as a suitable tool for routine monitoring.

HEADER: Effluent Toxicity Testing: Sublethal Toxicity Testing

METHOD NAME: Multispecies Algae Growth Inhibition Test

AETE REPORT REFERENCE: 1.2.2

PURPOSE: Evaluate effects of effluent exposure on the growth of three freshwater algae species. Result is expressed as the concentration where growth of the most sensitive algal species is reduced by 25% (IC25). Results are useful to answer the question "Is there a measurable response?"

DESCRIPTION: The test is a development of an existing International Standards Organization "Algal microtest battery", which was modified by the Saskatchewan Research Council (SRC). The endpoint is fluorescence so growth of different types of phytoplankton (filamentous, colonial, or unicellular organisms) can be measured. The method is described in SRC (1996) Draft Protocol for Phytoplankton Microplate Growth Inhibition Test Using a Fluorescence Endpoint.

The test uses three different phytoplankton (e.g., green alga *Selenastrum capricornutum*, blue-green algae *Microcystis aeruginosa* and diatoms *Nitzschia* sp). Tests are conducted on microplates under constant illumination in a temperature and humidity controlled chamber. After 45- 52 hours, fluorescence is measured and the most sensitive species is identified.

For QA/QC, detailed records of all aspects of the samples, test organisms, culture maintenance, test conditions, equipment and test results are validated and kept by the laboratory. Reference toxicant testing must be conducted with all species used in the test. Successive reference toxicant data are plotted on a control chart to ensure the performance of the test organisms.

Test requirements include an incubator with light, temperature and humidity control, a fluorescence reader, algal culture facilities and a source of high quality deionized/distilled water. Technical personnel must be skilled in botany, algal culture, axenic technique and bioassays. The test requires <1 L of effluent.

LIMITATIONS: The test may be invalid if the receiving waters are toxic to the test organisms. Effluents and receiving waters must be filtered before testing, which may modify toxicity. A major shortcoming is the lack of a standard test method - only the draft protocol is available. While the multi-species phytoplankton growth inhibition test was the most sensitive assay evaluated in AETE 1.2.2, the *Selenastrum* test is preferred due to the availability of a standard test method.

COST: Test is not currently available from commercial laboratories.

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Not recommended as a suitable tool for routine monitoring.

HEADER: Effluent Toxicity Testing: Sublethal Toxicity Testing

METHOD NAME: Microtox Chronic Test

AETE REPORT REFERENCE: 1.2.2

PURPOSE: To evaluate effects of effluent exposure on light production by the naturally luminescent marine bacteria, *Vibrio fischeri*. The result is expressed as the concentration where light output is reduced by 25% (IC25). The test results can be used to answer the question "Is there a measurable response?"

DESCRIPTION: The chronic test is a development of the acute test, using the same species and the same incubator/test system. The Microtox chronic test is described in the manufacturer's handbook "Measuring Chronic Toxicity Using Luminescent Bacteria" (Microbics Corp. 1994).

A bacterial culture is incubated with concentrations of the effluent at 27 °C. After 22 hours, the amount of light output of the bacteria is measured.

For QA/QC, detailed records of all aspects of the samples, test organisms, test conditions, equipment and test results are validated and kept by the laboratory. A reference toxicant is used to establish the validity of effluent toxicity data. Successive reference toxicant data are plotted on a control chart. If results are within expected limits, the performance of the batch of test organisms is ensured.

LIMITATIONS: The test organism is a marine bacteria, with little environment relevance to Canadian mining environments. The salinity of samples must be adjusted with the addition of NaCl (2% v/v) which may alter toxicity.

COST: NA (Test not recommended).

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Not recommended as a suitable tool for routine monitoring.

HEADER: Effluent Toxicity Testing: Sublethal Toxicity Testing

METHOD NAME: Mutatox Test

AETE REPORT REFERENCE: 1.2.2

PURPOSE: To evaluate the potential of effluent samples to cause a mutation. The result is not related to exposure concentration but is expressed as "yes" or "no". The test results can be used to answer question "Is there a measurable response?".

DESCRIPTION: The Mutatox uses a mutant strain of the Microtox organism *Vibrio fischeri*, which becomes luminescent when it undergoes a mutation to the wild type. The Mutatox test is described in the manufacturer's handbook "Mutatox Genotoxicity Test" (Microbics Corporation 1995).

A volume of sample is mixed with a vial of Mutatox Medium and dilutions are incubated in the analyzer. Exposures take place with and without the presence of the enzymatic activation solution S-9. Light output readings are taken after 16, 20 and 24 hours. A positive genotoxic response is defined as a light output greater than twice the control level. The sample is considered as genotoxic if a positive response is obtained in two consecutive dilutions.

LIMITATIONS: The test organism is a marine bacteria, with little environment relevance to Canadian mining environments. Test results are of an "all or none" format - either mutagenic or non-mutagenic and are difficult to compare to results of other tests. The salinity of samples must be adjusted with the addition of NaCl (2% v/v) which may alter the behavior of the sample.

COST: N/A

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Not recommended as a suitable tool for routine monitoring.

HEADER: Effluent Toxicity Testing: Sublethal Toxicity Testing

METHOD NAME: Nematode survival, maturation and growth test

AETE REPORT REFERENCE: 1.2.2

PURPOSE: Evaluate effects of effluent exposure on the survival, maturation and growth of the roundworm species *Panagrellus redivivus*.

DESCRIPTION: Nematodes are a significant component of the benthic fauna and the toxicity assay with this species is described in Samoiloff (1990). Result is expressed as the concentration where growth is reduced by 25% (IC25). If mortality is significant, it may be possible to calculate the lethal concentration for 50% of the test population (an LC50).

The assay involves the exposure of second stage juveniles to a range of effluent concentrations. During the 4 day test period, the juveniles pass through two other stages and become adults. At the end of the test, the number of survivors is recorded and the individuals are stained and measured. The length of the animal indicates its growth and stage of maturation. For the test to be considered valid, there must be > 890% survival in the controls and >40% of the control organisms must develop into adults.

The protocol / design of the assay has a major fault, encountered during testing with mining effluents. The number of animals remaining after staining were less than the recorded number of survivors. Animals may be lost during heating/evaporation step.

LIMITATIONS: Not recommended due to flaw in the test method.

COST: N/A

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Not recommended as a suitable tool for routine monitoring.

HEADER: Effluent Toxicity Testing: Sublethal Toxicity Testing

METHOD NAME: Viability of rainbow trout embryos.

AETE REPORT REFERENCE: 1.2.1, 1.2.2, 4.1.2

PURPOSE: Evaluate effects of effluent exposure to an early life stage of fish. Result is expressed as the concentration where embryo viability is reduced by 25% (LC25). The test results can be used to answer question "Is there a measurable response?".

DESCRIPTION: The only fish early life stage test for a considerable portion of Canada. The test method is Environment Canada EPS 1/RM/28. Biological test method: early life stage toxicity tests using salmonid fish (rainbow trout, coho salmon, or Atlantic salmon). Environment Canada 1996.

Newly fertilized salmonid eggs are exposed to a range of concentrations of an effluent for 7 days. Test exposure solutions are renewed every day. Dead embryos are counted and removed during the test. At the end of the test, surviving embryos are counted. Control viability must be ≥70% for the test to be acceptable.

For QA/QC, detailed records of all aspects of the samples, source of eggs and milt, test conditions, equipment and test results are maintained by the laboratory. A reference toxicant is used to establish the validity of effluent toxicity data. Successive reference toxicant data are plotted on a control chart to ensure the performance of the batch of test organisms.

Test requirements include a temperature controlled exposure system, bioassay equipment (exposure containers) and a source of clean non-chlorinated water. The laboratory must be able to obtain trout/salmon eggs and milt on a year-round basis. Technical personnel must be skilled in handling fish embryos and in bioassays. The test requires approximately 80-90 L of effluent.

LIMITATIONS: The test may be invalid if the receiving waters are toxic to the test organisms. It is difficult to obtain viable rainbow trout eggs and/or milt at certain times of the year. The test requires a large quantity of effluent and is more costly than other tests.

COST: $1735 (average from six CAEAL/MEF accredited labs -EEM Toxicology Expert Working Group).

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Not Recommended as a suitable tool for routine monitoring.

HEADER: Sediment Toxicity

METHOD NAME: Survival and growth of the freshwater midge (*Chironomus riparius*).

AETE REPORT REFERENCE: 4.1.3

PURPOSE: To evaluate effects of sediment samples on the reproduction of a chironomid. Result is expressed as "toxic" in comparison with a reference or upstream site. Effects can also be expressed as the difference (in %) with response in controls or reference sediments. The test response can be used to answer the question "Is there a measurable response?"

DESCRIPTION: Chironomids are often used to test sediments in the US and Canada. The test method is described in Environment Canada 1996. Test for Growth and Survival in Sediment Using Larvae of Freshwater Midges (*Chironomus tentans* Or *Chironomus riparius*) (Draft Method).

Ten second (*C. tentans*) or first (*C. riparius*) instar organisms are exposed to a sediment sample and bioassay water. Losses of overlying water due to evaporation are replaced during the test. After 10 days, the sediment is sieved and the surviving animals are counted, dried and weighed. Endpoints are survival and average growth. For a valid test, there must be >70% survival in the control sediment and mean growth must be ≥0.6 g (*C. tentans*) or ≥0.2 g (*C. riparius*).

For QA/QC, detailed records of all aspects of the samples, test organisms, test conditions, equipment and test results are kept by the laboratory. Control exposures with clean sediment are performed at the same time as sample tests. Testing of reference toxicants is done in "water only" exposures and the data plotted on a control chart. If individual results are within expected limits, the performance of the batch of test organisms is ensured.

LIMITATIONS: Test requirements include a temperature controlled room, bioassay equipment (exposure containers), invertebrate culture facilities and a source of clean non-chlorinated water. Technical personnel must be skilled in invertebrate biology and culture and in bioassays.

COST: Approximately $600

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Recommended as a suitable tool for routine monitoring.

HEADER: Sediment Toxicity

METHOD NAME: Survival and growth of the amphipod *Hyalella azteca*

AETE REPORT REFERENCE: 4.1.1, 4.1.3

PURPOSE: Evaluate effects of sediment samples on the survival and growth of an amphipod (small crustacean). Result is expressed as "toxic" in comparison with a reference or upstream site. Effects can also be expressed as the difference (in %) with response in controls or reference sediments. The test response can be used to answer the question "Is there a measurable response?"

DESCRIPTION: A widely used sediment test in Canada. The test method is described in Environment Canada 1996. Summary of Test Conditions for the *Hyalella azteca* 14-Day Sediment Survival and Growth Test (Draft Method).

Fifteen individuals (aged 1 -10 days) are exposed to sediment samples and bioassay water. Mortalities are monitored daily throughout the test. After 14 days, the sediment is sieved and the surviving animals are counted, dried and weighed. Endpoints are survival and average growth.

For QA/QC, detailed records of all aspects of the samples, test organisms, test conditions, equipment and test results are kept by the laboratory. Control exposures with clean sediment are performed at the same time as sample tests. Testing of reference toxicants is done in "water only" exposures and the data plotted on a control chart. If individual results are within expected limits, the performance of the batch of test organisms is ensured.

Sediment tests with *Hyalella* during the AETE field programs demonstrated this species to be sensitive to mine discharge.

Test requirements include a temperature controlled room, bioassay equipment (exposure containers), invertebrate culture facilities and a source of clean non-chlorinated water. Technical personnel must be skilled in invertebrate biology and culture and in bioassays.

LIMITATIONS: Sediment toxicity tests more expensive than characterization of benthic invertebrate community. *Hyalella* is not a tolerant species. Under lethal conditions for the organism, growth becomes irrelevant and cannot be used to measure relative differences in toxicity.

COST: $600

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Recommended as a suitable tool for routine monitoring.

Toolbox no.: 9.3

HEADER: Sediment Toxicity

METHOD NAME: Survival and reproduction of the oligochaete *Tubifex tubifex*.

AETE REPORT REFERENCE: 4.1.1, 4.1.3

PURPOSE: Evaluate effects of sediment samples on the reproduction of an oligochaete (segmented worm). Result is expressed as "toxic" in comparison with a reference or upstream site. Effects can also be expressed as the difference (in %) with response in controls or reference sediments. The test response can be used to answer the question "Is there a measurable response?"

DESCRIPTION: The test is described in the American Society for Testing and Materials 1992 Book of Standards: "Standard Guide For Conducting Sediment Toxicity Tests With Freshwater Invertebrates" ASTM E 1384-94a.(Vol. 11.04 Section 11).

Tubifex individuals are exposed to equal amounts of sediment and bioassay water. Sexually mature individuals (aged 8 weeks) are introduced and incubated for 28 days. The production of cocoons indicates reproduction of the organisms. At the end of the test, the sample is sieved and the number of surviving adults, the number of full and empty cocoons, the number of young < 500 mm and the number of young > 500 mm are counted as measurements of survival and reproduction.

For QA/QC, detailed records of all aspects of the samples, test organisms, test conditions, equipment and test results are kept by the laboratory. Control exposures with clean sediment are performed at the same time as sample tests. Testing of reference toxicants is done in "water only" exposures and the data plotted on a control chart. If individual results are within expected limits, the performance of the batch of test organisms is ensured.

Test requirements include a temperature controlled room, bioassay equipment (exposure containers), invertebrate culture facilities and a source of clean non-chlorinated water. Technical personnel must be skilled in invertebrate biology and culture and in bioassays.

LIMITATIONS: Test less sensitive than *Hyalella*. Toxicity tests are more expensive than characterization of the benthic community.

COST: $400-800

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Not recommended as a suitable tool for routine monitoring but may be appropriate as a substitute or additional test in site specific studies.

HEADER: Sediment Toxicity

METHOD NAME: Microtox Solid Phase Test

AETE REPORT REFERENCE: 4.1.1

PURPOSE: To evaluate effects of sediments on light production by the naturally luminescent marine bacteria, *Vibrio fischeri*. The result is expressed as the % decrease in light output. The test response can be used to answer the question "Is there a measurable response?"

DESCRIPTION: The solid phase test is a variant of the acute test, using the same species, the same incubator/test system and the same exposure period. The Solid-Phase test is described in the manufacturer's handbook "Microtox Manual. A Toxicity Testing Handbook. Volume II: Detailed Protocols (Microbics Corp. 1992).

The Microtox test is a rapid screening bioassay kit, which measures toxic effects on the light output of a standardized luminescent bacterial culture. In the solid-phase version, the bacteria are exposed to a suspension of the sample, the suspension is filtered, and the light output of the bacteria is measured. This is a rapid assay since the duration of the entire test is only 30 minutes. Results are normalized with respect to a clean reference sediment, one with similar physical characteristics to the test sample.

For QA/QC, detailed records of all aspects of the samples, test organisms, test conditions, equipment and test results are validated and kept by the laboratory. Control tests with clean sediment and reference toxicant tests are performed at the same time as sample tests are tested to validate the performance of the batch of test organisms.

LIMITATIONS: The test is less sensitive than whole organism tests with *Hyalella* and *Tubifex*. The test responded to field samples with intermediate to severe toxicity, but not to differences between reference, near-field and far-field stations.

COST: $270

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Not recommended as a suitable tool for routine monitoring.

HEADER: Biological Monitoring

METHOD NAME: Phytoplankton – Community Composition

AETE REPORT REFERENCE: 2.3.2.

PURPOSE: To evaluate the use of phytoplankton as an effective and meaningful biomonitoring tool for the Canadian mining industry.

DESCRIPTION: Approaches that were evaluated for use in studies include:

1) community canonical analysis
Algae are enumerated and classified using a microscope.

2) size distribution
This method is based on the enumeration and measurement of algae in a phytoplankton sample.

3) pigment analysis
Water samples are filtered and the filters extracted with ethanol or acetone.

4) phytochelatin analysis
Phytochelatins dosage can be measured using HPLC chromatography. The preparation of samples for chromatography involves several steps.

5) diatom deformities
Most deformities compose asymmetrical development of the valves. These observations can be done with a normal microscope or more subtle abnormalities have been observed with SEM microscope.

6) tests based on community induced tolerance.
In this method, the capacity of plankton communties subjected to elevated metal concentrations to increase their tolerance is exploited to detect contamination exposure.

LIMITATIONS: All of these approaches need field testing to verify their applicability. Considerable natural variability.

COST: Detailed costs are not available.

Was Tool Tested in AETE Field Program? No.

AETE RECOMMENDATION: Not recommended as a suitable tool for routine monitoring.

HEADER: Biological Monitoring

METHOD NAME: Periphyton – Community Composition.

AETE REPORT REFERENCE: 2.3.2.

PURPOSE: To evaluate the use of periphyton as a biomonitoring tool.

DESCRIPTION: The following methods were examined:

1) Dissimilarity index, community diversity and species evenness
Changes in taxonomic composition can be used to monitor the presence of metal contamination.
2) Niche center gradient analysis
This method calculates a niche center index to indicate the relationship of a diatom species to an environmental gradient of metals.

LIMITATIONS: Considerable natural variability. Field testing did not identify patterns related to mine effluent exposure. There is no standardized protocol.

COST:

1) Dissimilarity index, community diversity and species evenness
Total per sample: ~ $200 to $400

2) Niche center gradient analysis
Total per sample: ~ $150 to $250

Was Tool Tested in AETE Field Program? No.

AETE RECOMMENDATIONS: Not recommended as a suitable tool for routine monitoring.

HEADER: Biological Monitoring

METHOD NAME: Macrophyte - Community Structure.

AETE REPORT REFERENCE: 2.3.2.

PURPOSE: To compare plant communities associated with good water quality to those of a known degraded system. Such a survey over a large contamination gradient can be done fairly rapidly by biologists trained to recognize plant species.

DESCRIPTION: Emergent and submerged plants are identified in the field or are carefully collected and transported, to be identified by a qualified biologist. A list of macrophytes presence/absence, a rough count of the species number and the species encountered can be done.

LIMITATIONS: Since comparison with a healthy environment is the basis of the method, the ideal reference site must be similar in all respects (except for the presence of metal contamination and acidity due to mine activities). Other associated effects of mine activities, such as high turbidity and suspended solids, irregular water levels, rough substrates and poor nutrient levels can also have severe impact on inhabitants of the affected water bodies. There is no standard approach or protocol for this methodology.

COST: The cost of the field survey will be proportional to the dimensions of the Study Area and Reference Area. At least 2 technicians will be required, of which one must be a biologist or a technician trained to recognize macrophyte species. Ability to do scuba diving would also be an asset. The compilation of the data, the statistical analyses and their interpretation would be preferably done by the biologist who has done the field survey.

Was Tool Tested in AETE Field Program? No.

AETE RECOMMENDATIONS: Not recommended as a suitable tool for routine monitoring.

HEADER: Biological Monitoring

METHOD NAME: Biochemical Indicators in Aquatic Plants

AETE REPORT REFERENCE : 2.3.2

PURPOSE: To determine if there are biochemical indicators in plants that can be reliably correlated to indicate exposure to mining effluent.

DESCRIPTION: Various biochemical parameters have been measured in aquatic plants and studied to determine if concentrations or activity varies in response to exposure to mining effluent. These indicators include phytochelatins, enzymes and pigments. Phytochelatins are metal binding proteins found in the plant kingdom with a role similar to metallothionein in animals. They have been studied extensively in terrestrial plants but not in aquatic species. Enzyme activity and pigments (e.g. chlorophyll and carotenoids) can be extracted and measured by HPLC.

LIMITATIONS: There is a lack of standardized protocols available for collection and analysis of these endpoints. There is no demonstrated relationship between exposure and biochemical activity in the plants.

COST: variable depending on parameters measured

Was Tool Tested In AETE Program? no

AETE REPORT RECOMMENDATION: Not recommended for routine monitoring programs.

HEADER: Biological Monitoring

METHOD NAME: Use of mollusc growth as effects-monitors.

AETE REPORT REFERENCE: 2.3.1

PURPOSE: To evaluate the use of molluscs as effects-monitors for the Canadian Mining Industry.

DESCRIPTION: Molluscs as effects-monitors would monitor metal-induced effects such as the measurement of growth to estimate effects at the organismal level, and the measurement of condition to estimate effects at the population level.

LIMITATIONS: Further field testing is recommended before using molluscs as effects-monitors. Availability of molluscs for sampling and collection needs to be established before incorporating them into a long-term national biomonitoring program. Numerous abiotic and biotic measurements must be made in conjunction with metal concentrations in molluscs to interpret effectively the field results. Metal-induced effects in molluscs such as changes in growth are not well established and these types of responses should be used with caution.

COST: Costs are associated with animal collection, beginning and end-of-test measurements, deployment and retrieval activities, and chemical analyses. Costs range from $16,000 U.S. to $40,000 U.S. depending on whether two experienced practitioners are hired to guide the work, or a team are hired to conduct the entire study. These costs do not include travel and are dependent on the remoteness of collection and retrieval sites.

Was Tool Tested in AETE Field Program? No.

AETE RECOMMENDATION: Mollusc growth is not recommended as a routine monitoring tool.

HEADER: Biological Monitoring

METHOD NAME: Use of Benthic Macroinvertebrate fitness parameters to determine mining related impacts.

AETE REPORT REFERENCE: 2.1.5

PURPOSE: To evaluate the use of benthic macroinvertebrates as a group to measure the impact(s) of mine effluents within aquatic systems. Eight benthic macroinvertebrate fitness parameters were reviewed as indicators of benthic macroinvertebrate community health.

DESCRIPTION: Benthic invertebrates were evaluated for use as monitoring tools to measure the impacts of mine effluents within aquatic systems. The following eight macroinvertebrate population-level fitness parameters were reviewed for use as indicators of mining effects:

1. density (total number of individuals per sample),
2. size (e.g. head capsule width, body length),
3. condition (measured as a ratio of individual weight per unit head-width (mg/mm)),
4. fecundity (number of eggs per female),
5. adult emergence (e.g. the number of animals that successfully moult to adulthood, the timing of emergence),
6. distributional (behavioural) changes,
7. morphological deformities (e.g. mouthpart deformities such as missing/extra teeth),
8. fluctuating asymmetry (measured as the absolute difference between morphological traits (e.g. antennae segment length) on the right and left side of the body.

LIMITATIONS: The distribution and abundance of macroinvertebrates within ecosystems can be affected by a variety of abiotic and biotic factors other than water quality. Benthic macroinvertebrates do not respond to all impacts. Variation in distribution and abundance of macroinvertebrates can vary naturally. Macroinvertebrates, although relatively sedentary, can drift/crawl from one location to another.

COST: Not available.

Was Tool Tested in AETE Field Program? No.

AETE RECOMMENDATION: Benthic macroinvertebrates fitness parameters are not recommended for routine monitoring.

HEADER: Biological Monitoring

METHOD NAME: Benthic Community Descriptors.

AETE REPORT REFERENCE: 2.1.3. and field surveys

PURPOSE: To characterize the composition of benthic invertebrate communities.

DESCRIPTION: There are several ways to characterize benthic community composition including (1) total abundance, (2) species richness, (3) diversity indices, (4) biotic indices, (5) functional feeding groups, and (6) similarity indices.

Differences in community descriptors between reference and exposure areas are typically used as evidence of an effect of mine effluent. Advantages of using community descriptors include: 1) total abundance and richness are well established as descriptors, 2) diversity and biotic indices are also popular, 3) similarity indices summarize the overall differences in composition between communities at reference locations, and 4) test sites using a single number. They also require no pre-conceived assumptions of the nature of the effect and vary only in one direction. Functional feeding groups can assist in evaluating whether ecosystem function has been impaired.

LIMITATIONS: Total abundance and number of taxa may be unresponsive to slight degradation. Diversity and biotic indices respond primarily to organic enrichment, not necessarily effluent from mines. Functional feeding groups have not been well demonstrated to respond to effluent from mines. Similarity indices do not assist in interpreting the biology of observed effects.

COST: The costs of using any of these indices are associated with the regular costs of intepreting benthic community data. Costs for benthic community reports typically range between $2K and $5K.

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Benthic community descriptors are recommended as monitoring tools in a routine monitoring program.

HEADER: Biological Monitoring

METHOD NAME: Artificial substrates for benthos sample collection.

AETE REPORT REFERENCE: 2.1.1

PURPOSE: To review the use of artificial substrates for collection of benthic invertebrate samples, and to evaluate the utility and limitations of this method as a cost-effective environmental monitoring tool for the Canadian mining industry. Four classes of artificial substrates were evaluated for potential use in environmental monitoring for the mining industry.

DESCRIPTION: (See report for details)

1. Rock-filled Basket (or bag)
2. Beak Trays
3. Rock-filled Trays
4. Multiplate samplers

LIMITATIONS: They do not collect a representative sample of the indigenous benthos at the site, but rather select for mobile, drift-prone species of hard substrata. They indicate only the water quality during the colonization period, and do not integrate long-term effects. They do not effectively monitor the effects of sediments or sediment-bound toxicants and require two field trips to deploy and retrieve
Situations where artificial substrates could be used include: (1) water bodies with very deep or turbid water, (2) water bodies with soft or unstable bottoms of sand, mud or organic ooze, (3) water bodies with unbroken bedrock bottoms or bottoms of large boulders and, (4) rivers with torrential currents. Three kinds of artificial substrates that may be used in the mining industry are: Rock-filled baskets (or bags), Beak trays, and multiplate samplers.

COST: Cost of an artificial substrate sampling survey is approximately $4,740 based on a field survey of 5 sites with 5 replicates, excluding preparation, travel, sample processing and reporting.

Was Tool Tested in AETE Field Program? No.

AETE RECOMMENDATION: Artificial substrates should only be used for environmental monitoring on rivers or lakes that cannot be sampled using traditional methods. Their use is not recommended for routine monitoring.

HEADER: Biological Monitoring

METHOD NAME: Rapid Assessment Procedures in Benthic Invertebrate Monitoring

AETE REPORT REFERENCE: 2.1.2, 2.1.3

PURPOSE: To evaluate the use of rapid assessment procedures as an effective and meaningful biomonitoring tool for the Canadian mining industry.

DESCRIPTION: Multi-metric-rapid assessment procedures are designed to quickly identify water quality problems associated with point-source and non-point source pollution and to document long-term changes in environmental conditions within a region. They are based on comparisons between surveyed sites and clean reference sites that are taken as representative of the natural condition in the absence of human influence. These methods summarize results of site surveys in a way that can be easily understood by non-specialists such as managers, politicians and the concerned public. Many rapid assessment procedures combine a number of metrics (i.e. number of taxa, number of individuals, EPT richness etc.) into a single index that expresses the overall condition of the site. These procedures reduce costs by reducing benthic invertebrate sampling intensity and using simple, qualitative measures of benthic community composition (metrics) to compare study sites against regional reference sites.

LIMITATIONS: Rapid assessment procedures are not statistically based and are too insensitive for use in routine mining monitoring, but they may occasionally be useful for confirmation of severe impairment.

COST: Not provided

Was Tool Tested in AETE Field Program? No.

AETE RECOMMENDATIONS: Not recommended as a suitable tool for routine monitoring.

HEADER: Biological Monitoring

METHOD NAME: Evaluation of Biochemical Indicators in Fish

AETE REPORT REFERENCE: 2.2.3

PURPOSE: A literature review was undertaken to evaluate if biochemical indices in fish are useful as a measure of effects from mining activities.

DESCRIPTION: Fish contain a range of biochemical level enzymes, proteins, ions and other variables that are linked to important functions including growth, reproduction, immunity and respiration. Other indices including lipids, carbohydrates and blood ion status can be influenced by stresses or feeding status. AETE Report #2.2.3 (Table 1) provides a good summary of potential biochemical indices, their functions and responses to environmental changes. In general, biochemical indices respond rapidly to external conditions, therefore, may have some value as early warning systems. However, the link between changes in the level of most biochemical indices and the health of the individual or fish population is not established. Therefore, as a monitoring tool they have low ecological relevance. Furthermore, most indices do not show a specific response to metals but also respond to a host of other variables. Therefore, meaningful data interpretation is confounded in field situations. One possible exception is inhibition of ALAD activity by lead.

LIMITATIONS: Many tests not routinely available in commercial laboratories. Many indicators do not exhibit specific responses to only metals, therefore, interpretation to link to mining activity is speculative. Collection and analysis requires highly trained personnel.

COST: Varies widely depending on test.

Was Tool Tested In AETE Field Program? No

AETE RECOMMENDATION: Biochemical indicators are not recommended for use in a routine mining monitoring program.

HEADER: Biological Monitoring

METHOD NAME: Evaluation of Histopathology in Fish

AETE REPORT REFERENCE : 2.1.2

PURPOSE: Histopathology is the morphological evaluation of microscopic alterations seen in diseased organs and tissues. It is used in the field in an attempt to define the cause of death in fish die-offs and to examine the association between lesions and their causes.

DESCRIPTION: Fish tissues destined for histopathological examination must be properly sampled and preserved immediately upon death of the fish. Formalin is the most common preservative. Small fish (< 4 cm) can be preserved whole, medium size fish (4-10 cm) can be preserved by opening the abdomen. In larger fish it is necessary to remove all organs. Processing samples in the laboratory is a multi-step process that takes approxomately 24 hrs. Tissues are dehydrated, embedded in wax and sectioned on a microtome. The sections are placed on glass slides and stained. The tissue section is then examined by a pathologist under a microscope. Professional training requires several years including basic medical or veterinary medicine, apprenticeship training in biology and finally certification. Fish histopathology has a bias toward the effects of metals on gills compared to other organs. QA/QC in histopathological studies should occur during slide preparation and pathological interpretation. There are relatively few qualified individuals in Canada skilled in pathological evaluation.

LIMITATIONS: Several are noted by the authors. Not all toxicants have histopathological endpoints. Few toxicants leave a distinct fingerprint. Tissue examination is subjective and dependent upon previous experience of the examining pathologist. Changes in histopathology cannot necessarily be used to infer population level-effects. There is a lack of baseline histopathological data at mining sites in Canada. There is limited commercial (private) expertise although several universities and government labs will provide this service at a cost.

COST: approximately $150 to $225 per fish for full examination and interpretation.

Was Tool Tested In AETE Field Program? Yes

AETE RECOMMENDATION: Due to a lack of consistent response in the 1995 field survey and the AETE decision to not proceed with additional field studies, **AETE is unable to make recommendations on the use of histopathology of fish tissues in a routine mine monitoring program.**

HEADER: Biological Monitoring

METHOD NAME: Fish Organ Size

AETE REPORT REFERENCE: 2.2.3

PURPOSE: To evaluate organ level measurements and response to metals as a potential tool for examining environmental effects from mining activities.

DESCRIPTION: Exposure to contaminants can result in swelling or atrophy of some tissues. The relative size of a specific organ can be expressed as a percent of the total fish size (weight). The two most common indices are the Liver Somatic Index (LSI) or Gonadal Somatic Index (GSI) which are a measure of energy and reproductive energy investment, respectively. Tissue or cellular level examination of tissues is considered under histopathology (Toolbox Summary #3.4). Organs should be carefully excised from fresh fish and weighed. Freezing or preservation may interfere with size and/or weights. Gonad size will vary substantially depending on reproductive status of the fish. There may be significant variability between specimens such that adequate sample sizes (> 20 per species and sex) should be collected. Effect of metals on LSI and GSI not well established but decreases may be most common response. Indirect effects of metals on LSI or GSI may be manifest through changes in trophic structure and food availability. These indices are considered ecologically relevant.

LIMITATIONS: Few major limitations. Season of collection is important and must be standardized between areas and studies for meaningful comparison of results. GSI probably only useful for females, unless gross changes taking place in males. Organ weights should be collected accurately and carefully. Appropriate time should be allocated for these measurements but can be completed by most biologists. Changes in relative organ size may not be specific response to metal exposure, therefore, interpretation should be cautious.

COST: Difficult to discern because indices generally collected during general fish measurements. May increase examination time by 10-15 min per fish.

Was Tool Tested In AETE Field Program?: Relative liver and gonad sizes were measured in the field programs.

AETE RECOMMENDATION: Liver and gonad somatic indices are recommended as suitable tools in a routine monitoring program.

HEADER: Biological Monitoring

METHOD NAME: Fish Growth.

AETE REPORT REFERENCE: 2.2.3

PURPOSE: To evaluate effects at all levels of biological organization, particularly fish populations.

DESCRIPTION: The most complete description of study design and methods for an AFS are given in EC/DFO (1995). Shuter (1990) and Sandstrom (1996) give general descriptions of AFSs, while Munkittrick and M^cCarty (1995) review study-design considerations.

Usually one or two species with appropriate life-history characteristics are used for the studies. The key variables measured on fish from exposed and reference locations are age, size (length, weight), gonad weight (and fecundity in females), and growth (size at age), condition (weight at length). Liver weight and age at maturity are often added as other variables, but age at maturity can be difficult to estimate without considerable effort.

Differences in AFS variables between reference and exposed fish are used as evidence of an effect due to mine effluent. The nature of the observed differences can be used to diagnose the specific cause of the effect. The advantage of an AFS is that it is the most practical population-level tool available. The survey can be targeted to forage species and have limited effects on game or commercial species. Interpretive frameworks provide diagnostic potential.

LIMITATIONS: Based on the experience of the pulp and paper EEM and previous AETE field testing, the AFS will not be successful at every site. The effects of destructive sampling can be as great or greater than those from anthropogenic activities. Destructive sampling in areas with a local recreational fishery is usually unpopular if the species used in the assessment is a sport fish. Recreational exploitation of sport fish can confound interpretations.

COST: $10-30K exclusive of data analysis and report writing.

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: Fish growth is recommended as a suitable tool in routine monitoring.

HEADER: Biological Monitoring

METHOD NAME: Abundance (CPUE) of Key Fish Species.

AETE REPORT REFFRENCE: 2.2.3

PURPOSE: To evaluate the effects of mine effluent on fish populations.

DESCRIPTION: Population abundances are directly estimated, but because absolute abundances are difficult to estimate, abundance indices (i.e., catch-per-unit effort, CPUE) are often used.

Fish are collected from reference and exposed locations. Differences in abundance between reference and exposed population are used as evidence of effluent-related effects.

EVS (1997) list several reports and papers that describe approaches to estimating abundances of key species in various habitats.

LIMITATIONS: Variances in estimated abundances are often large. Consequently, high numbers of fish from many locations are often required to obtain reasonably precise estimates of abundance and to provide adequate statistical power for detection of effects. Many capture methods will be destructive.

COST: Costs were not quoted in EVS (1997), but costs of the survey will generally be covered with the AFS as long as the targeted species for CPUE work is also targeted for an AFS.

Was Tool Tested in AETE Field Program? Yes.

AETE RECOMMENDATION: CPUE data are recommended to be included as incidental information that can be useful with other monitoring data but should not be used alone.

HEADER: Biological Monitoring

METHOD NAME: Fish Community Survey (FCS).

AETE REPORT REFERENCE: 2.2.3

PURPOSE: To evaluate the effects of mine effluent on fish populations.

DESCRIPTION: Fish are collected from several exposed and unexposed locations. Differences in the composition of the fish communities, or in indices of composition between exposed and unexposed communities are used as evidence of an effect.

Community assessments include qualitative (presence/absence) and quantitative (estimated abundances of each species) surveys. Community-level assessments are considered more biologically relevant than population assessments because they characterize effects on all species (game, forage) and reflect effects on a higher level of organization.

Fausch *et al.* (1990) provide a good review of approaches to the use of fish community surveys in environmental monitoring. Fish community surveys are generally not applied in site-specific assessments. Rather, they are more generally used for regional assessments that may include sites influenced by mines or metals.

LIMITATIONS: Large numbers of fish are required from large numbers of sites to give a survey with adequate statistical power to detect effects.

COST: Not provided.

Was Tool Tested in AETE Field Program? No.

AETE RECOMMENDATION: Fish community surveys (FCSs) are not recommended for general use in monitoring programs as a stand alone tool. However, species composition may provide useful additonal information on general habitat quality.